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THE CONE BIOPSY—HYSTERECTOMY TIME INTERVAL RELATED TO WOUND INFECTION

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Abstract. A group of 99 patients, who underwent total hysterectomy because of carcinoma in situ of the cervix between 3 and 57 days after cervical cone biopsy were compared with a control group of 130 patients who were subjected to hysterectomy because of uterine fibroids with no preceding biopsy. A significantly increased frequency of wound infections was found in the cone-biopsy-hysterectomy group, 23.3% as compared with 8.2% in the control group. Furthermore a significant increase in febrile morbidity was found in the cone-biopsy-hysterectomy group, 31.3% as compared with 10.8% in the control group. In the cone-biopsy-hysterectomy group wound infections only occurred when the time interval between the two operations was between 3 and 14 days. With an interval of 15 days or more no wound infections were found. No relationship could be established between the febrile morbidity rate and the cone-biopsy-hysterectomy interval. Other complications were similar in both groups. The most frequent complications were urinary tract infections and paresis. No deaths occurred. It is recommended that hysterectomy should not be performed until at least two weeks after a cone biopsy of the cervix, in order to avoid the risk of wound infection which was 4 times commoner than in our control series.

At the obstetrical and gynaecological department of Øresundshospitalet, Copenhagen, we were under the impression that an increased number of wound infections occurred in patients who had been subjected to cone biopsy of the cervix prior to hysterectomy. The following analysis was performed to study this problem.

In 1958 Osoba (10) published a study of 38 patients in whom hysterectomy had been carried out from 2 days to 8 weeks after a cone biopsy of the cervix. He found a post-operative febrile morbidity of 52.6% against 21.8% in a control group who had not had a previous cervical biopsy. A strikingly increased number of febrile complications were found when the cone-biopsy-hysterectomy interval was less than 10 days, including one death from staphylococcal septicaemia. Osoba holds that the cone biopsy may cause a subclinical parametritis, and that a subsequent hysterectomy may spread this infection throughout the operation wound. The author therefore recommends that the hysterectomy be postponed until 4 to 6 weeks after the cone biopsy.

A number of later studies report similar results. Cavanagh & Rutledge (2) found febrile morbidity in 42.4% of a cone-biopsy-hysterectomy group against 14.5% of a control group. Malinak et al. (9) reported a febrile morbidity in 58.9% against 42.9% of the control group. If the cone-biopsy-hysterectomy interval was less than 19 days there was a significant increase in febrile episodes, wound infections and abscesses. All serious complications including one death from septicaemia occurred in this group, and the authors conclude that the interval between cone biopsy and hysterectomy should be at least 3 weeks. *Doran & Shie* (3) found febrile morbidity in 45% of a cone-biopsy-hysterectomy group; all the patients who were operated on 7 to 14 days after a cone biopsy were febrile. Laubach & McGarity (7) reported a febrile morbidity in 54.6% against 39.0% in the control group and Kaufman et al. (5) found 52.0% against 23.8%. Kummert (6) has analysed a series of 167 patients who underwent hysterectomy after previous cone biopsy. He found febrile morbidity in 22.4% when the cone-biopsy-hysterectomy interval was 3 to 14 days. This morbidity was significantly higher than was the case if the interval was less than 48 hours (5.5%) or between 2 and 4 weeks (8.3%). The author concludes that a previous

Table I. *Wound infection in patients following hysterectomy with or without previous cone biopsy of the cervix*

	Total no of patients	No of patients with infection in the abdominal wound	No of patients with infection in the vaginal vault	Total no. of patients with wound infection
Hysterectomy + cone biopsy	99	16 (16._)	7 (7.1)	_3 (_3._)
Hysterectomy alone	130	7 (5.5)	1 (0.8)	8 (6._)

cone biopsy of the cervix does not increase the post hysterectomy morbidity if the correct time interval is chosen between the two operations. Williams et al. (11) in a study from the Mayo Clinic, have compared 941 patients, who had cone biopsy with frozen-section diagnosis followed immediately by hysterectomy with 61 patients who had hysterectomy more than 48 hours after the cone biopsy After abdominal hysterectomy febrile morbidity was found in 56% of the latter group while the morbidity rate was 30 in the former group and 2 in a control group who had hysterectomy only After vaginal hysterectomy the morbidity in the various groups did not differ significantly No serious or fatal complications occurred in any of the groups.

MATERIAL AND METHOD

The series consists of 99 consecutive patients, who in the period April 1967 – October 1969 underwent cone biopsy of the cervix and then between 3 and 57 days later had hysterectomy for carcinoma in situ of the cervix. The preliminary diagnosis was based on smears and small biopsies from the cervix, and these were followed routinely by cone biopsy of the cervix in order to exclude invasive growth. To avoid the patient suffering any emotional strain as result of any delay the total hysterectomy was performed approximately one week after the cone biopsy if possible

The control group consists of 130 consecutive patients, who in the same period of time underwent total hysterectomy for uterine fibroids, without previous cone biopsy

The preoperative procedure was the same in both groups. On the evening before the operation a neomycin-bacitracin pessary was placed high in the vagina In the operating theatre the vagina was cleaned out with hexachlorophene emulsion and then rinsed with sterile water. The abdominal skin was disinfected twice using iodine In every case abdominal hysterectomy was performed.

All patients in the cone biopsy group had a vaginal cuff excised. During the operation the uterine arteries were ligated with nylon, but otherwise only plain and chromic catgut was used inside the abdomen. The vaginal vault was closed with interrupted chromic catgut sutures, the abdominal wound with continuous chromic catgut suture in the peritoneum, interrupted nylon sutures in the fascia, 3–4 nylon sutures through the skin and subcutaneous fat and metal clips in the skin. The clips were removed on the 7th day and the sutures in the skin 10 days after the operation. The mean duration of the operation for both groups was 1 hours.

The technique for the cone biopsy was as follows The cervix was painted with Schiller's solution and an incision made with a cold knife beyond the area which failed to stain. The circular incision was directed toward the internal os and the cone removed. After this, two Sturmdorf sutures were placed anteriorly and posteriorly in the cervix and if necessary a haemostatic suture was inserted on each side of the cervix. All sutures were of chromic catgut. An iodoform gauze pack was placed in the vagina and removed the following day Before the hysterectomy the cervix was controlled to be sure of a clean cervical wound.

The average age of the patients in the cone biopsy group was 43.8 years (25–61 years) and for the control group 46.3 years (31–68 years). No prophylactic antibiotics were used and appendectomy was not performed as routine

During this study special attention was paid to wound infection, but febrile morbidity and other complications were also recorded. Wound infection in the abdominal wound was defined as an abscess which required opening, and in the vaginal wound as an abscess or unusual induration accompanied by purulent vaginal discharge Febrile morbidity is defined as a rectal temperature of 38 C or over for day or more

RESULTS

Table I shows that among 99 patients who had cone biopsies prior to hysterectomy _3 developed wound infections. Of the 130 patients in the control group 6._ had wound infections. This difference is statistically significant (3–4 times the standard error)

In Table II the wound infection rate is related to the number of days between cone biopsy and hysterectomy Nearly all the patients had their hysterectomy done within 3 weeks of the cone biopsy Only 3 patients were operated on later the maximum being 5 days after cone biopsy As the table shows, the infected cases were equally scattered over the first 14 days. After this time no wound infection was seen

Table III demonstrates an increased febrile morbidity in the cone biopsy group (31.3%) compared with the control group (10.8%). This difference is significant (greater than twice the standard error). No relationship was found between the febrile morbidity rate and the cone-biopsy-hysterectomy interval.

Other complications were similar in both groups. The most frequent complications were urinary tract infections and pneumonia. No deaths occurred.

DISCUSSION

The results found in this series agree with those reported by other investigators. A significant increase in wound infections and febrile morbidity was demonstrated in a group of patients who underwent hysterectomy between 3 and 57 days after a cone biopsy as compared with a control group who had a hysterectomy only. I the cone biopsy group wound infections only occurred when the hysterectomy was performed 3–14 days aft r the biopsy. With a cone-biopsy-hysterectomy interval of 15 days or more no wound infection was found, but the number of patients in this group is too small to allow any firm conclusions. No *correlation* could be established between febrile morbidity and the cone-biopsy-hysterectomy interval.

The incidence of wound infection in the control group (6.2%) is higher than in other Danish studies following hysterectomy. Becker & Kristoffersen (*1*) report wound infection in 3%, Hornbæk et al. (4) in 4% and Lefevre (8) in 4.4%. The high wound infection rate in the control group may be due to the fact that 3 of the patients with infection in the abdominal wound had an infected necrotic fibroid polyp protruding through the cervix at the time of hysterectomy.

Table II. *Cone biopsy-hysterectomy-interval related to wound infection rate*

Interval in days	No. of patients	No. of patients with wound infection
3–7 days	56	14 (25.0)
8–14	35	9 (25.7)
15–57	8	0
Total	99	23 (23.2 %)

Table III. *Febrile morbidity in patients following hysterectomy with or without previous cone biopsy of the cervix*

	Total no. of patients	No. of patients showing febrile morbidity
Hysterectomy + cone biopsy	99	31 (31.3)
Hysterectomy alone	130	14 (10.8%)

Moreover it is remarkable that the wound infections most frequently occurred in the abdominal wounds.

CONCLUSION

The above study shows that when total hysterectomy is performed 3–14 days after a cone biopsy of the cervix there is a risk of wound infection which is approximately four times higher than is found in a control group which has had hysterectomy alone. Therefore it is recommended that hysterectomy be postponed until at least 2 weeks after the cone biopsy.

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DISLOCATION OF THE NASAL SEPTAL CARTILAGE IN THE NEWBORN

Aetiology Spontaneous Course and Treatment

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Abstract. Following dislocation of the lower edge of the nasal septal cartilage from the furrow in the premaxilla and vomer (Figs. 1 2) the cartilaginous outer nose is often twisted and the support is poor (Figs. 4 *a*, *b*). In 7% of the cases no deviation is evident unless compression-test is carried out (Fig 4 *b*). This procedure is recommended in the routine examination of newborn infants. If twisting is found the infant should be examined by a specialist in ear, nose and throat diseases. 141 cases of dislocation of the nasal septal cartilage were found in a series of 9 707 living newborn infants (1,45%) (Fig 5) However, in a series of 907 newborn infants all examined rhinologically 29 cases were found (3.19%) (Fig 6). Two thirds of the cases are apparently caused by trauma during pregnancy and the early stages of labour They are equally distributed between right and left. One third of the cases are caused by trauma solely during internal rotation, such as the L O A-presentation causes a dislocation of the inferior edge to the right, in the R O A-presentation to the left (Figs. 10, 11 12) The condition occurred significantly more commonly in first-born infants but the reason for this was not evident from the analyses of the data. It was also more common in multiparae if second-stage of labour was prolonged beyond 15 min (Table VIII) Therefore it is suggested that the second stage should not exceed 15 min in multiparae The dislocation does not reduce spontaneously The results obtained after reduction according to the method of Metzenbaum (Fig. 4 *c*) are good (Fig. 13), and the procedure can be carried out under local anaesthesia.

The nasal septal cartilage forms the anterior portion of the septum The front upper edge forms the nasal bridge in the midline from the nasal bones to the apex, while it is fixed posteriorly to the perpendicular plate of the ethmoid. The lower edge lies in a furrow in the maxillary spine, the premaxilla and the front part of the upper edge of the vomer (Fig. 1). It is attached by strong strands of connective tissue from the periosteum of the irregular edges of the furrow to the perichondrium. Some of these fibres cross the midline (4 16). It is generally accepted that the nasal septum is rarely in the midline, and that in some cases of distorted septum, symptoms appear such as nasal stenosis and/or an increased tendency to infections of the upper and lower respiratory tract. Therefore, it is of importance to find and if possible to avoid the aetiological factors causing dislocation of the septal cartilage and also to find suitable methods of treatment. This study is concerned with *dislocations of the nasal septal cartilage* (septum dislocation) in the newborn, its probable aetiology spontaneous course and treatment.

REVIEW OF THE LITERATURE

Septum dislocation in the newborn was first described in 1936 by Metzenbaum (9) and later by several others (1 4 6, 8, 11 14 15). Following dislocation of a greater or lesser portion of the lower edge of the septal cartilage from the furrow it sinks to the bottom of the cavity alongside the crest, and a deviation is formed in the opposite direction posteriorly

Support of the cartilaginous part of the nasal bridge becomes poor and at the same time a deviation occurs in which the apex points in the opposite direction to the dislocation. The columella becomes distorted and the nasal apertures asymmetric. In addition the nasal aperture on the dislocated side is flattened. The edge of the pre

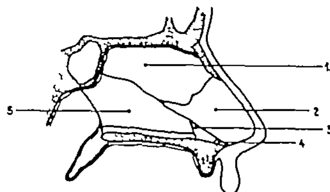


Fig 1 The nasal septum (after Mosher). *1* Perpendicular plate of the ethmoid, septal cartilage *3* premaxilla *4* spine of maxilla, *5* vomer

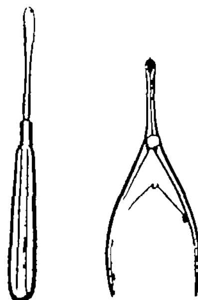


Fig 3

maxilla is often prominent on the opposite side of the septum (Fig. 2) The lower edge of the dislocated cartilage can frequently be felt with a narrow instrument (8)

Aetiology

It has been reported that septal dislocations can take place during pregnancy It is assumed that it can be caused by long sustained pressure, for instance by a foetal hand or a uterine fibromyoma (14)

A certain amount of adaption of the supple head of the child to the birth canal occurs during labour The distance between the apex of the nose and the occiput is nearly the same as that between the occiput and the chin (occipito-mental) and these are the longest diameters of the head (9 3) Therefore it must be presumed that the soft cartilaginous part of the nose is subjected to particular pressure during birth as it projects from the face (9) The importance of birth trauma as a probable aetiological factor was first reported by Metzenbaum (9) who found 2 cases of dislocation in a series of ?00 births. Cottle et al. (3) found severe nasal deformities in 5 8 to 10 % of newborn infants. However they detailed neither the size of population investigated nor the incidence of septum dislocations. The hypothesis is based on the impression that there is an apparent connection between the frequency of certain foetal presentations (and consequently the direction of rotation during labour) and the frequency of certain directions of dislocations. Thus Briant (1) and Steiner (14) points out the apparent agreement between the frequency of the foetal presentation Left Occiput Anterior (LOA) which should occur in 70–80% of births in OA-presentations, and the more frequent occurrence of deviation of the newborn child's nose to the right (70–85%) Briant (1) and Steiner (14) state that this predominant deviation to the right with LOA is associated with a corresponding dislocation of the lower edge of the septal cartilage into the left nasal cavity With Right Occiput Anterior-presentation (ROA) one should find a dislocation of the lower edge to the right. Steiner (14) expects a higher frequency of dislocations in presentations associated with a longer arch of rotation.

This hypothesis is also supported by the impression that deformities are more frequent in first born infants and by the observation that the frequency is lower in faces that have flat noses (14)

However some cases are assumed to be caused by trauma during the first stage of labour before internal rotation occurs (14)



Fig 2 Diagram of the commonest findings in dislocation to the right.

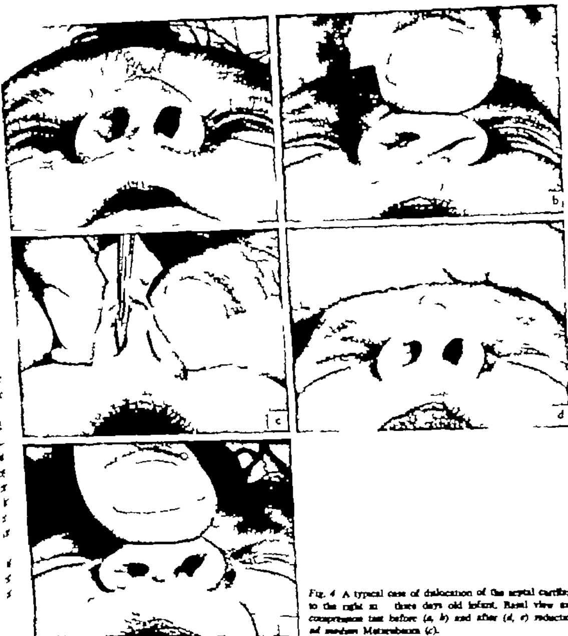


Fig. 4. A typical case of dislocation of the septal cartilage to the right in a three days old infant. Basal view and compression test before (*a*, *b*) and after (*d*, *e*) reduction ad modum Metzenbaum (*c*).

Spontaneous course

Neuman (10) by examining a number of children of different age groups, demonstrated that the frequency and severity of septum deformities increases with increasing age. However this was based on cross-sectional population studies rather than repeated observations on the same group of children during infancy. Neuman (10) presumed that the increasing frequency and severity resulted from the original trauma causing a permanent developmental defect. Kirchner (7) reported three cases of severe deviations of the whole outer nose and septum which could not be corrected shortly after birth, but complete spontaneous remission occurred 2 weeks to 2 months after birth.

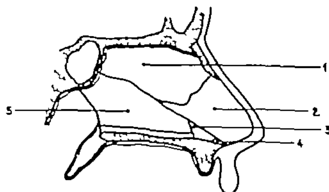


Fig 1 The nasal septum (after Mosher). *1* Perpendicular plate of the ethmoid, septal cartilage *3* premaxilla, *4* spine of maxilla, *5* vomer

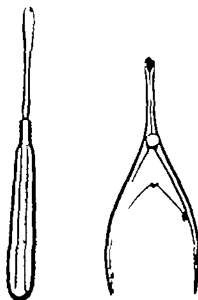


Fig 3

maxilla is often prominent on the opposite side of the septum (Fig. 2) The lower edge of the dislocated cartilage can frequently be felt with a narrow instrument (8)

Aetiology

It has been reported that septal dislocations can take place during pregnancy It is assumed that it can be caused by long sustained pressure for instance by a foetal hand or a uterine fibromyoma (14)

A certain amount of adaption of the supple head of the child to the birth canal occurs during labour The distance between the apex of the nose and the occiput is nearly the same as that between the occiput and the chin (occipito-mental) and these are the longest diameters of the head (9 3) Therefore it must be presumed that the soft cartilaginous part of the nose is subjected to particular pressure during birth as it projects from the face (9) The importance of birth trauma as a probable aetiological factor was first reported by Metzenbaum (9) who found 2 cases of dislocation in a series of 200 births. Cottle et al. (3) found severe nasal deformities in 5 8 to 10% of newborn infants. However they detailed neither the size of population investigated nor the incidence of septum dislocations. The hypothesis is based on the impression that there is an apparent connection between the frequency of certain foetal presentations (and consequently the direction of rotation during labour) and the frequency of certain directions of dislocations. Thus Briant (1) and Steiner (14) points out the apparent agreement between the frequency of the foetal presentation *Left Occiput Anterior* (LOA) which should occur in 70–80% of births *in OA-presentations, and the more frequent oc*currence of deviation of the newborn child's nose to the right (70–85%) Briant (1) and Steiner (14) state that this predominant deviation to the right with LOA is associated with a corresponding dislocation of the lower edge of the septal cartilage into the left nasal cavity With Right Occiput Anterior-presentation (ROA) one should find a dislocation of the lower edge to the right. Steiner (14) expects a higher frequency of dislocations in presentations associated with a longer arch of rotation.

This hypothesis is also supported by the impression that deformities are more frequent in first born infants and by the observation that the frequency is lower in faces that have flat noses (14)

However some cases are assumed to be caused by *trauma during the first stage of labour before* internal rotation occurs (14)



Fig Diagram of the commonest findings in dislocation to the right.

Table I. *Distribution of dislocations of the septal cartilage in the material 1965–70*

	N	% of all cases	Confidence limits ( )	
Dislocation to the right	77	55	46–63	0 05
Dislocation to the left	64	45	37–54	0 05
Total	141			

strate any significant difference in the frequency of dislocations to the right and to the left ($p = 0.05$).

No information is available as to which side the outer nose was deviated in 28 of the 141 cases. The distribution of the deviation of the outer nose in the remaining 113 cases is shown in Table II. Thus in 7% of the cases no deviation of the outer nose was found and in 4% of the cases it took place to the same side as the dislocation of the inferior edge of the cartilage.

Aetiology

In order to find significant aetiological factors the material has been analysed with regard to the occurrence of factors during the pregnancy and labour that could be considered pathogenic. The occurrence of these has been compared with that in a normal series consisting of all labours taking place within the period under study

(*a*) *Complications during pregnancy, manipulations and surgical procedures.* No significant difference was found in the occurrence of these factors in the series and the control group (Table III)

(*b*) *Primi and multipara.* When the series was compared with the control group, it was found that the incidence of the disorder was highly significantly more common in *primiparae than in multiparae (Table IV).*

(*c*) *The age of the mother.* A significant lower frequency of the age group 35 years or more was found in the material than in the control group (Table V).

(*d*) *The duration of the first and second stage.* No significant difference was demonstrated in the duration of total labour in the series as compared with the control group. There was no significant difference in the in-

Table II. *Deviation of the external nose in comparison to the direction of the dislocation of the septal cartilage in 113 registered cases*

	N	%
Deviation in opposite direction to the dislocation	100	89
No deviation	8	7
Deviation to the same side as the dislocation	5	4
Total	113	100

Table III. *Complications and operations during pregnancy*

Figures within parentheses denote percentages

	Material	Confidence limits (p 0 05)	Control[a]
Hemorrhage during pregnancy	5 (3 5)	(1.1–8.2)	218 (3.06) 1
Preeclampsia	10 (7.1)	(3.5–12.8)	571 (8 01) 1
Eclampsia	0	(0–2 6)	13 (0.18) 1
Imminent, premature labour	2 (1 4)	(0.2 5 0)	113 (1.58) 1
X-ray in early pregnancy	0	(0–2 6)	45 (0 63) 1
Contracted pelvis	2 (1 4)	(0 2–5 0)	59 (0 82) 1
Height of mother <150 cm	0	(0–2 6)	30 (0 42) 1
Excess weight of mother before pregnancy (>10)	11 (7.8)	(4 0–13.7)	46 (9 20) 8
Hydramnios	0	(0–2 6)	66 (0 92) 1
Prolonged pregnancy	8 (5 7)	(2 5–11 0)	505 (7.08) 1
Previous Caesarean section	2 (1 4)	(0 2 5 0)	174 (2 44) 1
Shirodkar operation	0	(0–2 6)	4 (0 05) 1
External version	1 (0 7)	(0–3.9)	11 (2.20) 8

1 = total number of mothers 1965–69· 7124
8 = 500 consecutive cases.

cidence of Caesarean section prior to labour in the two groups (Table VI)

(*e*) *The duration of the second stage.* A second stage of 15 min or more occurred significantly more frequently in the affected cases than in the control group (Table VII). The *frequency of second stage phase having a duration* of 15 min or more in primi- and multipara with OA-presentations can be seen from Table VIII. The frequency was the same in the primipara but significantly higher in the multipara of the series than of the control group.

(*f*) *Complications during labour and special factors regarding the newborn.* The frequency of complications during labour was not significantly higher in the affected cases than in the control group. On the other hand moulding was significantly less common in affected cases than in the control group and the difference was highly significant (Table IX, X).

Table IV. *Parity*

Figures within parentheses denote percentages

	Material	Control[a]	χ^2-test
Primipara	80 (56 74)	4 151 (42.76) 2	$\chi^2 = 10\ 488$
Multipara	61 (43 26)	5 560 (57.25) 2	0 005 p 1 <0 001
Total	141	9 711	

2 total number of mothers 1965–70 9 711

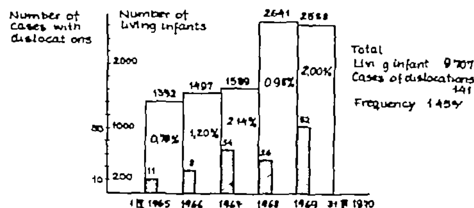


Fig. 5 Frequency of dislocations of the septal cartilage 1965–70

A study of the spontaneous course of septum dislocations detected post natally is not available as far as is known to the authors.

Treatment

Several authors recommend the reduction of the dislocation as soon as possible (2 6 8 9 11) and at the latest 3 weeks after birth (9) A method for reducing these dislocations was published by Metzenbaum (9) and later in detail by Klaff (8) The cartilaginous distal part of the nose is held by means of gauze between the thumb and first finger It is lifted and at the same time the dislocated side is pressed with one of the fingers in towards the midline At the same time the lower edge of the septum is lifted with an elevator (Fig. 3) which is inserted under the free edge The reduction can often be heard when the cartilage slips into position The support of the apex is often improved after the reduction, but quite frequently a slight twisting of the nose remains. This however disappears spontaneously within the first few weeks (8) The method has later been used by several authors (6 11)

PRESENT INVESTIGATION

Method

During the period 1 April 1965 – 14 Sept. 1969 all the newborn infants in the Department of Obstetrics and Gynaecology University Hospital, Odense in whom the staff of the gynaecological-obstetric department found twisting of the outer nose together with instability of the cartilaginous portion were examined at the Department of Otorhinolaryngology If the examination disclosed septum dislocation this was reduced according to the method of Metzenbaum (9) (Figs. 4 a, b c d) under local anesthesia using Lidocain gel 5% Follow-up examinations were carried out on the 4th day and one and four months after reduction.

As a special group a total of 907 nonselected newborn infants were all examined rhinologically in the period 14 Sept. 1969 to 31 March 1970. During the period 14 Sept. to 31 Dec. 1969 reduction and follow-up examination was carried out as mentioned above and in the period 1 Jan. to 31 March 1970 reduction was not carried out on any dislocation found. A follow-up examination was made 3 months after birth.

Material and results

The number of living children born in the hospital and the frequency of septum dislocations during the various years under study and also in the total series (1.45) can be seen from Fig. 5 The number of newborn infants examined and the frequency of septum dislocations in the period 14 Sept. 1969 to 31 March 1970 (3.19%) can be seen from Fig. 6. The difference found is significant ($p < 0.001$)

The distribution of septum dislocations to right (55%) and left (45) respectively in the material from 1965 to 1970 is shown in Table I It was not possible to demon-

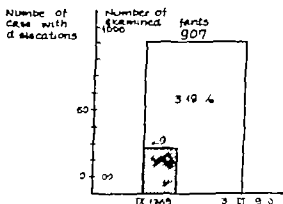


Fig. 6 Frequency of dislocations of the septal cartilage Group 14 Sept. 1969 31 March 1970.

I the period 1 April 1965 t 1 July 1969 this carried out by Stoksted (15), Olesen (11) and Jarh (6).

Table X. *Condition of the newborn*

Figures within parentheses denote percentages

Condition	Material	Control[a]		χ^2-test
Girls	77 (54.60)	235 (47.00)	B	$\chi^2 = 2.25$ (0.1 < p | n = 1 < 0.2)
Boys	64 (45.39)	265 (53.00)	B	
Weight at birth < 2 500 g	7 (4.96)	865 (8.91)	A	$\chi^2 = 2.21$ (0.1 < p | n = 1 < 0.2)
Weight at birth > 4 000 g	13 (9.21)	69 (13.80)	B	$\chi^2 = 1.68$ (0.1 < p | n = 1 < 0.2)
Moulding	19 (13.47)	125 (25.00)	B	$\chi^2 = 7.74$ (0.005 < p | n = 1 < 0.01)
Caput succedaneum	24 (17.02)	114 (22.80)	B	$\chi^2 = 1.86$ (0.1 < p | n = 1 < 0.2)

A = total number of living infants 1965–70 9 707. B = 500 consecutive cases.

outer nose to straighten spontaneously in contrast to septum dislocation. Unfortunately it was not possible to carry out follow-up examination of one of these cases.

In 13 cases reduction was performed according to the method of Metzenbaum and this resulted in the septum being returned to the midline in ten of the patients. While in the other three a slight bulging of a minor part of the lower edge of the septum cartilage remained. In these cases the cartilaginous part of the outer nose was still twisted. At the follow-up examination, on an average 3.9 months later, the outer nose was found to be straight in all cases.

The dislocation did not relapse in any case.

In one case a slight bulging of a part of the lower edge of the septum cartilage was found. Following reduction and at the control examination 4 and 30 days later the septum was in the midline. Of the previously mentioned three patients in whom the reduction to the midline was not complete, two straightened themselves spontaneously while a third continued to show a slight bulging of a small part of the septum.

As expected, a significant difference was found in the frequency of dislocations at the follow-up examination of the treated and untreated patients (Fig. 13).

Table XI. *Fetal presentations*

Figures within parentheses denote percentages

Presentation	Material	Control		χ^2-test
Left occiput ant.	86	5 811		
	(61.0)	(58.9)	3	
Right occiput ant.	39	2 471		
	(27.7)	(25.0)	3	$\chi^2 = 7.554$
Occiput post.	2	301		
	(1.4)	(3.0)	3	0.1 < p | n = 4 < 0.2
Breech	2	613		
	(1.4)	(6.2)	3	
Other presentations[a]	12	677		
	(8.5)	(6.9)	3	
Total	141	9 873		
Twins	0	320		p < 0.05
	(0)	(3.24)		
	(0–2.6)	(2.2–4.5)		

Other presentations

Occiput transverse	1	(0.70)	57	(0.57)	3
Sincipital	2	(1.41)	88	(0.89)	3
Brow	0		12	(0.12)	3
Face	1	(0.70)	19	(0.19)	3
Pubes arrest & conversion	1	(0.70)	18	(0.18)	3
Unregistered cephalic	7	(4.96)	451	(4.56)	3
Transverse lie & caesarean section	0		32	(0.32)	3

DISCUSSION AND CONCLUSIONS

The frequency of dislocation of the septal cartilage found in a series of 9 707 living newborn infants was 1.45%. However, in a series of 907 newborn infants who were all examined rhinologically it was 3.19% ($p < 0.001$). The frequency was thus higher than in the smaller series of Metzenbaum (9) (200 patients). The higher frequency found in the group of 907 newborn infants could partly be explained by the fact that dislocation will not be suspected in 7% of the cases where deviation of the outer nose is not present and partly by better examination techniques.

However, a septum dislocation should be suspected, when the nose twists to one side or the

Table XII. *Direction of the septal dislocation in LOA- and ROA-presentation*

Figures within parentheses denote percentages

Presentation / Direction of dislocation	Material	Confidence limits
LOA right	55 (64.0)	52.88–74.03
LOA left	31 (36.0)	25.97–47.12
ROA left	25 (64.1)	47.18–78.80
ROA right	14 (35.9)	21.20–52.82
		p < 0.05

Table V *Age of the mother*

Figures within parentheses denote percentages

Age (y)	Material	Control*	χ²-test
<18	4 (2.83)	3 7 (3.36)	
18–30	121 (85.81)	7 709 (79.38)	$\chi^2 = 7.300$
31 35	15 (10 63)	1 103 (11.35) 2	$0.05 < p \mid r = 3 < 0.1$
>35	1 (0.70)	572 (5.88)	
	141	9 711	

= total number of mothers 1965–70 9 711

Table VI *Duration of first and second-stage pains (total labour pains)*

Figures within parentheses denote percentages

Hours	Material	Control*	χ²-test
<1	118 (83.68)	406 (81.20) 8	
1 24	70 (14 18)	74 (14 80) 8	$\chi^2 = 0.741$
> 4	1 (0.70)	8 (1 60) 8	$0.6 < p \mid r = \quad < 0.7$
Cesarian section without pains	(1 41)	1 (2.40) 8	
	141	500	

8 = 500 consecutive cases

Table VII *Duration of second-stage pains*

Figures within parentheses denote percentages

Minutes	Material	Control*	χ²-test
>15	98 (69.50)	289 (57 80) 8	$\chi^2 = 5.817$
<15	43 (30.50)	11 (4 20) 8	$0.01 < p \mid r = 1 < 0.025$
	141	500	

8 = 500 consecutive cases.

(g) *Foetal presentations.* There was a tendency to a lower frequency of breech presentations in the series than in the control group, whereas all the other presentations seem to occur with equal frequency (Table XI).

(h) *Number of right and left dislocations in the LOA and ROA presentations* The material shows dislocation to the right in two thirds and to the left in one third of the infants born in LOA-presentation. This difference is significant ($p = 0.05$). Correspondingly there was dislocation to the left in two thirds and to the right in one third of the infants born in ROA presentation. Possibly because of the small number of cases this difference is not significant (Table XII).

Table VIII *Duration of second-stage pains >15 min in primi and multipara in OA-presentations*

Figures within parentheses denote percentages

	Material	Control*	χ²-test
Primipara			
>15	61 (87 14)	14 (90 68)	$\chi^2 = 0.4036$
<15	9 (1_86)	— (9.37) 9	$0.5 < p \mid \; = 1 < 0.6$
	70	236	
Multipara			
>15	_6 (47.27)	8? (31 06) 9	$\chi^2 = 4.643$
<15	29 (5_73)	182 (68.94) 9	$0.0_5 < p \mid \quad 1 < 0.05$
	55	64	

9 = 500 consecutive OA-presentations.

The spontaneous course and the results following reduction according to the method of Metzenbaum (Fig. 13)

In 16 cases of septum dislocation reduction was not performed. At the follow-up examination, on an average 3 months later, spontaneous reduction of the dislocation was not observed in any case In 14 cases the nose was straight while in one case the same deviation of the apex was found as that after labour There appears to be a pronounced tendency for the cartilaginous part of the

Table IX *Complications during labour*

Figures within parentheses denotes percentages

Complication	Material	Confidence limits (p 0 05)	Control*
Cervical rupture	0	(0– 6)	33 (0 46) 1
Vaginal rupture	4 (_83)	(0.8–7 1)	117 (1 64) 1
Perineal rupture	12 (8.51)	(4 5–14 5)	14 (4 16–5 66) 6
Precipitate delivery	0	(0– 6)	11 (0 4) 7
Abruptio placentae	0	(0– 6)	145 (03) 1
Rupture of the bag of waters >6 h before labour	6 (4 _5)	(1 6–9 1)	375 (5 ?6) 1
Funiculus feu circumflectens	39 (27 65)	(20 7 36 0)	1518 (20 91) 5
Imminent, fetal hypoxia	10 (7 09)	(3 5–1 8)	555 (7 64) 5
Artificial delivery	18 (1 76)	(7 8–19 5)	1371 (16 18) 4

1 = Total number of mothers 1965–69 7 1 4 4 total number of living infants 1965–70 9 707 5 total number of infants 1965–69 7 _57 6 = total number of mothers 1965–67 2910 7 = total number of mothers 1965–68 4 487

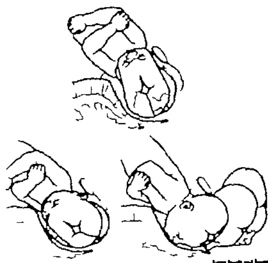


Fig. 9. The movements of the foetal head during internal rotation (Steele & Javert, 1942).

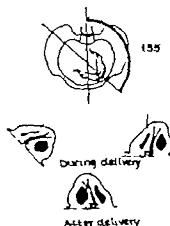


Fig. 11. The occurrence of dislocation of the septal cartilage during rotation in Right Occiput Anterior (ROA) presentation.

Possible nasal trauma during labour

From the moment when labour pains start and the descent is commenced the foetal head, and with this the projecting nose, is affected by innumerable trauma of varying directions and strength (Fig. 7). This continues during the further descent of the foetal head (Fig. 8). When the internal rotation commences, the foetal head including the nose, is affected by a well defined trauma, the direction of which is decided only by the foetal presentation in question (Fig. 9).

As OA-presentation comprises 89 % of all foetal presentations, and as the course of labour is best known in this presentation we chose to analyse this part of the series with regard to the possible connection between the direction of the septum dislocation and the foetal presentation.

In the LOA-presentation the internal rotation of the foetal head occurs, observed from the pelvic inlet, in a clockwise direction. The left nasal ala is thus pressed towards the anterior portion of the septal cartilage and then towards the right cheek. It is presumed that the inferior edge of the septum during this rotation is dislocated into the right side of the nose and that the outer nose swings to the left after labour (Fig. 10). Correspondingly the internal rotation in the



Fig. 10. The occurrence of dislocation of the septal cartilage during rotation in Left Occiput Anterior (LOA) presentation.

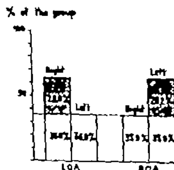


Fig. 12. An analysis of the distribution of the direction of dislocations in LOA and ROA presentations in the material.

other on direct vertical pressure (Fig. 4 b) This procedure is very easy to carry out and should be used in the routine examination of the new born.

(a) The complications and procedures during pregnancy under study occurred with the same frequency in the series as in the control group although one would expect such factors to cause major trauma to the foetal nose

(b) The infants of primipara were affected significantly more frequently This agrees with the observations of Steiner (14)

(c) It has not been possible to demonstrate a definite predominance of younger mothers in the material, within the age groups chosen.

(d e) The total length of the first and second stages of labour does not appear to influence the occurrence of dislocation. However a duration of second stage pains of 15 mm or more was more common in the material. It is a wellknown fact that the duration of the second stage is often greater in primiparae than in multiparae There fore the longer mean duration could be ascribed to the predominance of primiparae However the

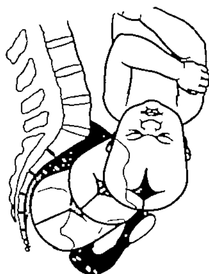


Fig 8 Further descent into the pelvis until engagement (Steele & Javert, 1942)

study demonstrates that the multiparae in the material were responsible for the longer duration of second stage.

(f) Labours during which a greater trauma to the foetus might be expected were not more frequent. A significantly lower frequency of moulding was the only factor which differed. This appears to demonstrate that lack of moulding presumably adds to nasal trauma during labour

There was no significantly higher frequency of heavy newborn infants.

(g) The different foetal presentations appear with equal frequency in the series and the control group with the exception of Breech-presentation, which was less common in the affected cases. The difference is not significant.

(h) The fact shown in this study that the inferior edge of the cartilage was dislocated into the right side of the nose in 55% of all cases and in nearly two thirds of the cases delivered in LOA presentation, is in contrast to previous reports. According to Briant (1) and Steiner (14) dislocation of the inferior edge to the left should be expected in 70–85% of the cases and should mainly appear after labour in LOA presentation. Correspondingly dislocation to the left appeared in nearly two thirds of the cases delivered in ROA-presentation in the present study while according to Steiner (14) the dislocation should appear to the right in these cases.

A thorough analysis of the course of labour is therefore of importance.

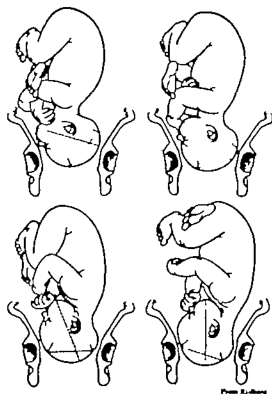


Fig 7 The figure shows the initial movements of the foetal head in the pelvic inlet, necessary for delivery in OA-presentation (Rydberg, 1954).

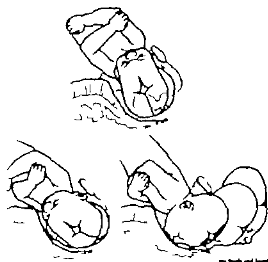


Fig. 9. The movements of the foetal head during internal rotation (Steele & Javert, 1942)

Possible nasal trauma during labour

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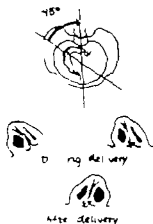


Fig. 10. The occurrence of dislocation of the septal cartilage during rotation in Left Occiput Anterior (LOA) presentation.

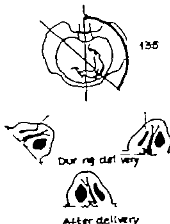


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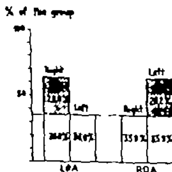


Fig. 12. An analysis of the distribution of the direction of dislocations in LOA and ROA presentations in the material.

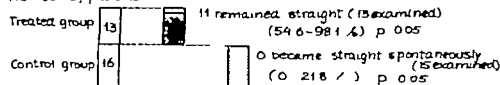


Fig. 13 The results following reduction ad modum Metzenbaum 14 Sept. 1969 31 March 1970.

ROA-presentation will occur as observed from the pelvic inlet, in an anticlockwise direction. The right nasal ala is thus pressed towards the septal cartilage and then towards the left cheek. It is presumed that during this the septum is dislocated into the left side of the nose, and that the outer nose swings to the right after birth (Fig. 11)

One would presume that the internal rotation was the cause of the dislocation in the two thirds of the cases in which it takes place to the side expected according to the direction of the internal rotation. However two infants with dislocation were delivered by Caesarean section before labour started, which shows that septum dislocation can take place during pregnancy Thus in these cases the internal rotation has no influence on the direction of the dislocation. It is probable that dislocation can also be caused by all the varying trauma other than the internal rotation that affect the foetal nose during labour Thus in these cases the internal rotation has no influence on the direction. If the figures from Table XII are now considered in the manner shown in Fig. 12 it is seen that the cases of dislocation distributed according to LOA and ROA-presentation partly occur in two identical groups, within which an equal number of dislocations to the right and left is found. Partly in two equal groups, the character of which is such that they only include cases of dislocation in which the dislocation occurs to the expected side taking into account the direction of the internal rotation.

Cases of dislocations occurring during pregnancy and the early stages of labour are caused by trauma from varying directions and may thus statistically be equally represented between the right and left. These cases can explain the two identical groups.

The trauma of the internal rotation has only one well defined direction and is the only one during pregnancy and labour the direction of which is determined by the presentation alone. This can explain the remaining two equal groups, consisting of one third of the cases, the direction of which is that to be expected from the foetal presentation (Figs. 10 11)

The influence of the length of rotation on the distribution of the dislocations in LOA and ROA

According to Ingerslev (5) the head of the foetus in the OA-presentation is in the first oblique diameter (right diameter) on entering the pelvis in two thirds of the cases. This diameter is shown in Figs. 10 and 11 This will result in a rotation of 45 degrees for the two thirds of the foetuses born in the LOA-presentation, while two thirds of the foetuses born in the ROA-presentation rotate 135 degrees. One would immediately assume that a longer rotation would give a greater risk of trauma and therefore a greater frequency of dislocations to the left in the ROA-presentation and a lower frequency of dislocations to the right in LOA-presentation would be expected. However the material shows that the frequency is equal (Table XII) Thus the length of rotation is of no importance and the theory of Steiner (14) could not be confirmed.

(f) It has been demonstrated that septum dislocations do not reduce spontaneously in contrast to twisting of the outer nose.

In a number of cases of deviation of the nasal septum, complications arise such as nasal stenosis and an increased tendency to infections of the upper and lower respiratory tract. As reduction probably does not result in additional trauma of any importance this should be carried out, and preferably within the first week of life The results following reduction according to the method of Metzenbaum are good, and it is easily performed under local anaesthesia. This is in agreement with the literature (6 8 9 11) But only continued study of the treated and untreated cases during growth can give the answer to the basic question. Should a dislocation of the nasal septal cartilage always be reduced in order to ensure normal physiological function of the nose?

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DISLOCATION OF THE NASAL SEPTAL CARTILAGE IN THE NEWBORN

Aetiology Spontaneous Course and Treatment

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Abstract Following dislocation of the lower edge of the nasal septal cartilage from the furrow in the premaxilla and vomer (Figs. 1 2) the cartilaginous outer nose is often twisted and the support is poor (Figs. 4 *a*, *b*). In 7% of the cases no deviation is evident unless compression-test is carried out (Fig 4 *b*). This procedure is recommended in the routine examination of newborn infants. If twisting is found the infant should be examined by specialist in ear nose and throat diseases. 141 cases of dislocation of the nasal septal cartilage were found in series of 9 707 living newborn infants (1.45%) (Fig 5) However in series of 907 newborn infants all examined rhinologically 29 cases were found (3.19) (Fig 6) Two thirds of the cases are apparently caused by trauma during pregnancy and the early stages of labour They are equally distributed between right and left. One third of the cases are caused by trauma solely during internal rotation, such as the L.O.A.-presentation causes dislocation of the inferior edge to the right, in the R.O.A.-presentation to the left (Figs 10 11, 12). The condition occurred significantly more commonly in first-born infants but the reason for this is not evident from the analyses of the data It is also more common in multiparae if second-stage of labour was prolonged beyond 15 min (Table VIII). Therefore it is suggested that the second stage should not exceed 15 min in multiparae. The dislocation does not reduce spontaneously The results obtained *after reduction according to the method of* Metzenbaum (Fig. 4 *c*) are good (Fig. 13), and the procedure can be carried out under local anaesthesia.

The nasal septal cartilage forms the anterior portion of the septum. The front upper edge forms the nasal bridge in the midline from the nasal bones to the apex, while it is fixed posteriorly to the perpendicular plate of the ethmoid. The lower edge lies in furrow in the maxillary spine, the premaxilla and the front part of the upper edge of the vomer (Fig. 1). It is attached by strong strands of connective tissue from the periosteum of the irregular edges of the furrow to the perichondrium. Some of these fibres cross the midline (4 16). It is generally accepted that the nasal septum is rarely in the midline, and that in some cases of distorted septum, symptoms appear such as nasal stenosis and/or an increased tendency to infections of the upper and lower respiratory tract. Therefore, it is of importance to find and if possible to avoid the aetiological factors causing dislocation of the septal cartilage and also to find suitable methods of treatment. This study is concerned with dislocations of the nasal septal cartilage (septum dislocation) in the newborn, its probable aetiology spontaneous course and treatment.

REVIEW OF THE LITERATURE

Septum dislocation in the newborn was first described in 1936 by Metzenbaum (9) and later by several others (1 4 6, 8, 11 14 15) Following *dislocation of a greater or lesser portion of the lower edge of the septal cartilage from the furrow* it sinks to the bottom of the cavity alongside the crest, and a deviation is formed in the opposite direction posteriorly

Support of the cartilaginous part of the nasal bridge becomes poor and at the same time a deviation occurs in which the apex points in the *opposite direction* to the dislocation. The columella becomes distorted and the nasal apertures asymmetric. In addition the nasal aperture on the dislocated side is flattened. The edge of the pre

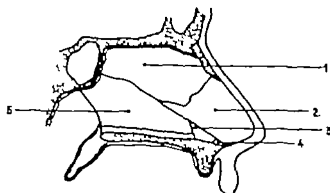


Fig 1 The nasal septum (after Mosher). *1* Perpendicular plate of the ethmoid, septal cartilage *3* premaxilla *4* spine of maxilla, *5* vomer

maxilla is often prominent on the opposite side of the septum (Fig. 2) The lower edge of the dislocated cartilage can frequently be felt with a narrow instrument (8)

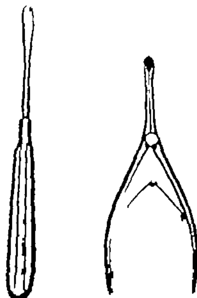


Fig 3

Aetiology

It has been reported that septal dislocations can take place during pregnancy It is assumed that it can be caused by long sustained pressure for instance by a foetal hand or a uterine fibromyoma (14)

A certain amount of adaption of the supple head of the child to the birth canal occurs during labour The distance between the apex of the nose and the occiput is nearly the same as that between the occiput and the chin (occipito-mental) and these are the longest diameters of the head (9 3) Therefore it must be presumed that the soft cartilaginous part of the nose is subjected to particular pressure during birth as it projects from the face (9) The importance of birth trauma as a probable aetiological factor was first reported by Metzenbaum (9) who found 2 cases of dislocation in a series of 200 births. Cottle et al. (3) found severe nasal deformities in 5.8 to 10% of newborn infants. However they detailed neither the size of population investigated nor the incidence of septum dislocations. The hypothesis is based on the impression that there is an apparent connection between the frequency of certain foetal presentations (and consequently the direction of rotation during labour) and the frequency of certain directions of dislocations. Thus Briant (1) and Steiner (14) points out the apparent agreement between the frequency of the foetal presentation Left Occiput Anterior (LOA) which should occur in 70–80% of births in OA-presentations, and the more frequent occurrence of deviation of the newborn child's nose to the right (70–85%) Briant (1) and Steiner (14) state that this predominant deviation to the right with LOA is associated with a corresponding dislocation of the lower edge of the septal cartilage into the left nasal cavity With Right Occiput Anterior-presentation (ROA) one should find a dislocation of the lower edge to the right. Steiner (14) expects a higher frequency of dislocations in presentations associated with a longer arch of rotation.

This hypothesis is also supported by the impression that deformities are more frequent in first born infants and by the observation that the frequency is lower in faces that have flat noses (14)

However some cases are assumed to be caused by trauma during the first stage of labour before internal rotation occurs (14)



Fig Diagram of the commonest findings in dislocation to the right.

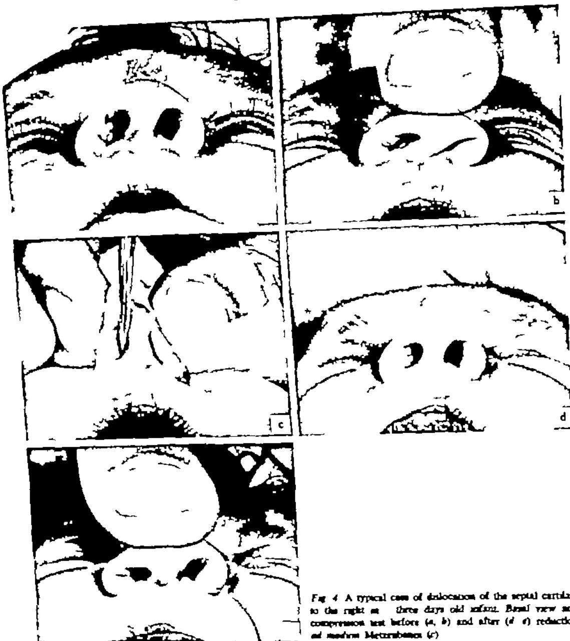


Fig. 4. A typical case of dislocation of the septal cartilage to the right in three days old infant. Basal view and compression test before (*a*, *b*) and after (*d*, *e*) reduction ad modum Metzenbaum (*c*)

Spontaneous course

Neuman (10) by examining a number of children of different age groups, demonstrated that the frequency and severity of septum deformities increases with increasing age. However this was based on cross-sectional population studies rather than repeated observations on the same group of children during infancy. Neuman (10) presumed that the increasing frequency and severity resulted from the original trauma causing a permanent developmental defect. Kirchner (7) reported three cases of severe deviations of the whole outer nose and septum which could not be corrected shortly after birth, but complete spontaneous remission occurred 2 weeks to 2 months after birth.

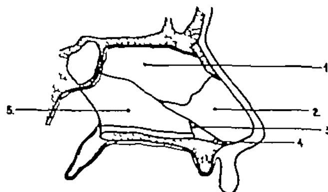


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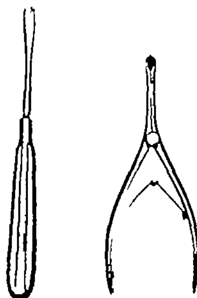


Fig 3

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Table I. *Distribution of dislocations of the septal cartilage in the material 1965–70*

	N	% of all cases	Confidence limits ( )	
Dislocation to the right	77	55	46–63	0.05
Dislocation to the left	64	45	37–54	0.05
Total	141			

strate any significant difference in the frequency of dislocations to the right and to the left ($p = 0.05$).

No information is available as to which side the outer nose was deviated in 28 of the 141 cases. The distribution of the deviation of the outer nose in the remaining 113 cases is shown in Table II. Thus in 7% of the cases no deviation of the outer nose was found and in 4% of the cases it took place to the same side as the dislocation of the inferior edge of the cartilage.

Aetiology

In order to find significant aetiological factors the material has been analysed with regard to the occurrence of factors during the pregnancy and labour that could be considered pathogenic. The occurrence of these has been compared with that in a normal series consisting of all labours taking place within the period under study.

(a) *Complications during pregnancy, manipulations and surgical procedures.* No significant difference was found in the occurrence of these factors in the series and the control group (Table III).

(b) *Primi and multipara.* When the series was compared with the control group, it was found that the incidence of the disorder was highly significantly more common in primiparae than in multiparae (Table IV).

(c) *The age of the mother.* A significant lower frequency of the age group 35 years or more was found in the material than in the control group (Table V).

(d) *The duration of the first and second stage.* No significant difference was demonstrated in the duration of total labour in the series as compared with the control group. There was no significant difference in the incidence of Caesarean section prior to labour in the two groups (Table VI).

(e) *The duration of the second stage.* A second stage of 15 min or more occurred significantly more frequently in the affected cases than in the control group (Table VII). The frequency of second stage pains having a duration of 15 min or more in primi- and multipara with OA presentations can be seen from Table VIII. The frequency was the same in the primipara but significantly higher in the multipara of the series than of the control group.

(f) *Complications during labour and special factors regarding the new-born.* The frequency of complications during labour was not significantly higher in the affected cases than in the control group. On the other hand moulding was significantly less common in affected cases than in the control group and this difference was highly significant (Table IX, X).

Table II. *Deviation of the external nose in comparison to the direction of the dislocation of the septal cartilage in 113 registered cases*

	N	
Deviation in opposite direction to the dislocation	100	89
No deviation	8	7
Deviation to the same side as the dislocation	5	4
Total	113	100

Table III. *Complications and operations during pregnancy*

Figures within parentheses denote percentages

	Material	Confidence limits ($p = 0.05$)	Control[a]
Hemorrhage during pregnancy	5 (3.5)	(1.1–8.2)	218 (3.06) 1
Preeclampsia	10 (7.1)	(3.5–12.8)	571 (8.01) 1
Eclampsia	0	(0–2.6)	13 (0.18) 1
Imminent, premature labour	2 (1.4)	(0.2–5.0)	113 (1.58) 1
X-ray in early pregnancy	0	(0–2.6)	45 (0.63) 1
Contracted pelvis	2 (1.4)	(0.2–5.0)	59 (0.82) 1
Height of mother < 150 cm	0	(0–2.6)	30 (0.42) 1
Excess weight of mother before pregnancy (> 10)	11 (7.8)	(4.0–13.7)	46 (9.20) 8
Hydramnios	0	(0–2.6)	66 (0.92) 1
Prolonged pregnancy	8 (5.7)	(2.5–11.0)	505 (7.08) 1
Previous Caesarean section	2 (1.4)	(0.2–5.0)	174 (2.44) 1
Shirodkar operation	0	(0–2.6)	4 (0.05) 1
External version	1 (0.7)	(0–3.9)	11 (2.20) 8

1 = total number of mothers 1965–69: 7124.
8 500 consecutive cases

Table IV. *Parity*

Figures within parentheses denote percentages

	Material	Control[a]	χ^2-test
Primipara	80 (56.74)	4 151 (42.78) 2	$\chi^2 = 10.448$
Multipara	61 (43.26)	5 560 (57.25) 2	$0.005 < p$ [illegible] < 0.001
Total	141	9 711	

2 total number of mothers 1965–70: 9 711

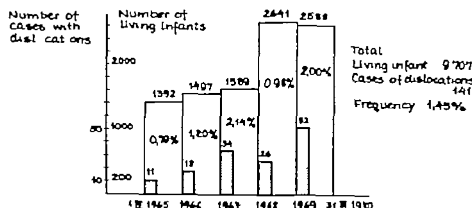


Fig 5 Frequency of dislocations of the septal cartilage 1965–70

A study of the spontaneous course of septum dislocations detected post natally is not available as far as is known to the authors.

Treatment

Several authors recommend the reduction of the dislocation as soon as possible (2, 6 8 9 11) and at the latest 3 weeks after birth (9) A method for reducing these dislocations was published by Metzenbaum (9) and later in detail by Klaff (8) The cartilaginous distal part of the nose is held by means of gauze between the thumb and first finger It is lifted, and at the same time the dislocated side is pressed with one of the fingers in towards the midline At the same time the lower edge of the septum is lifted with an elevator (Fig. 3) which is inserted under the free edge The reduction can often be heard when the cartilage slips into position The support of the apex is often improved after the re duction, but quite frequently a slight twisting of the nose remains. This, however disappears spon taneously within the first few weeks (8) The method has later been used by several authors (6, 11)

PRESENT INVESTIGATION

Method

During the period 1 April 1965 – 14 Sept. 1969 all the newborn infants in the Department of Obstetrics and Gynaecology University Hospital, Odense in whom the staff f the gynaecological-obstetric department found twisting of the outer nose together with instability of the cartilaginous portion were examined at the Department of Otorhinolaryngology If the examination disclosed septum dislocation this was reduced according to the method of Metzenbaum (9) (Figs. 4 *a*, *b* *c*, *d*) under local anesthesia using Lidocain gel 5 %. Follow-up ex aminations were carried out on the 4th day and one and four months after reduction.

As special group a total of 907 nonselected newborn infants were all examined rhinologically in the period 14 Sept. 1969 to 31 March 1970. During the period 14 Sept. to 31 Dec. 1969 reduction and follow-up examination was carried out as mentioned above and in the period 1 Jan. to 31 March 1970 reduction was not carried out on any dislocation found. A follow-up examination was made 3 months after birth.

Material and results

The number of living children born in the hospital and the *frequency of septum dislocations* during the arious years under study and also in the total series (1 45%) can be seen from Fig. 5 The number f newborn infants examined and the frequency of septum dislocations in the period 14 Sept. 1969 to 31 March 1970 (3 19%) can be seen from Fig. 6. The difference found is significant ($P < 0.001$)

The distribution of septum dislocations to right (55) and left (45) respectively in the material from 1965 to 1970 is shown in Table I. It was not possible to demon-

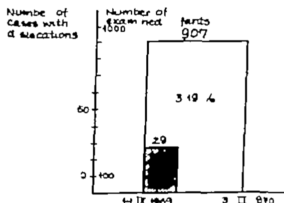


Fig 6 Frequency of dislocations f the septal cartilage. Group 14 Sept. 1969 31 March 1970

1 the period 1 April 1965 t 1 July 1969 this as carried out by Stoksted (15), Olesen (11) and Jazbi (6).

Table X. Condition of the newborn

Figures within parentheses denote percentages

Condition	Material	Control[a]	χ^2-test
Girls	77 (54.60)	235 (47.00) [b]	$\chi^2 = 2.25$ (0.1 < p < 0.2)
Boys	64 (45.39)	265 (53.00) [b]	
Weight of birth < 2 500 g	7 (4.96)	863 (8.91) [a]	$\chi^2 = 2.21$ (0.1 < p < 0.2)
Weight of birth > 4 000 g	13 (9.21)	69 (13.80) [b]	$\chi^2 = 1.68$ (0.1 < p < 0.2)
Moulding	19 (13.47)	125 (25.00) [b]	$\chi^2 = 7.74$ (0.005 < p < 0.01)
Caput succedaneum	24 (17.02)	114 (22.80) [b]	$\chi^2 = 1.86$ (0.1 < p < 0.2)

a total number of living infants 1965–70 9 707. b = 500 consecutive cases.

outer nose to straighten spontaneously in contrast to septum dislocation. Unfortunately it was not possible to carry out follow-up examination of one of these cases.

In 13 cases reduction was performed according to the method of Metzenbaum and this resulted in the septum being returned to the midline in ten of the patients while in the other three a slight bulging of a minor part of the lower edge of the septum cartilage remained. In nine cases the cartilaginous part of the outer nose was still twisted. At the follow-up examination, on an average 3.9 months later *the outer nose was found to be straight in all cases.*

The dislocation did not relapse in any case.

Table XI. *Fetal presentations*

Figures within parentheses denote percentages

Presentation	Material	Control	χ^2-test
Left occiput ant.	86 (61.0)	5 811 (58.9) [b]	
Right occiput ant.	39 (27.7)	2 471 (25.0) [b]	χ^2 7.554
Occiput post.	2 (1.4)	301 (3.0) [b]	0.1 < p < 0.2
Breech	2 (1.4)	413 (4.2) [b]	
Other presentations[a]	12 (8.5)	677 (6.9) [b]	
Total	141	9 873	
T[illegible]	0 (0) (0–2.4)	320 (3.24) (2.2–4.5)	0.05

Other presentations

	Material		Control		
Occiput transverse	1	(0.70)	57	(0.57)	[b]
Sincipital	2	(1.41)	88	(0.89)	[b]
Brow	0		12	(0.12)	[b]
Face	1	(0.70)	19	(0.19)	[b]
Pelvic breech & caesarean sect.	1	(0.70)	18	(0.18)	[b]
Unregistered cephalic	7	(4.96)	451	(4.56)	[b]
Transverse lie & caesarean section	0		12	(0.12)	[b]

In one case a slight bulging of a part of the lower edge of the septum cartilage was found. Following reduction and at the control examination 4 and 30 days later the septum was in the midline. Of the previously mentioned three patients in whom the reduction to the midline was not complete, two straightened themselves spontaneously while the third continued to show a slight bulging of a small part of the septum.

As expected, a significant difference was found in the frequency of dislocations at the follow-up examination of the treated and untreated patients (Fig. 13).

DISCUSSION AND CONCLUSIONS

The frequency of dislocation of the septal cartilage found in a series of 9 707 living newborn infants was 1.45%. However, in a series of 907 newborn infants who were all examined rhinologically it was 3.19% ($p < 0.001$). The frequency was thus higher than in the smaller series of Metzenbaum (9) (200 patients). The higher frequency found in the group of 907 newborn infants could partly be explained by the fact that dislocation will not be suspected in 7% of the cases where deviation of the outer nose is not present and partly by better examination techniques.

However, a septum dislocation should be suspected, when the nose twists to one side or the

Table XII. *Direction of the septal dislocation in LOA and ROA-presentation*

Figures within parentheses denote percentages

Presentation Direction of dislocation	Material	Confidence limits
LOA right	55 (64.0)	52.88–74.03
LOA left	31 (36.0)	25.97–47.12
ROA left	25 (64.1)	47.18–78.80
ROA right	14 (35.9)	21.20–52.82
		p 0.03

Table V *Age of the mother*

Figures within parentheses denote percentages

Age (y)	Material	Control*	χ^2-test
< 18	4 (2.83)	327 (3.36) [2]	
18–30	121 (85.81)	7 709 (79.38) [2]	$\chi^2 = 7.300$
31–35	15 (10.63)	1 103 (11.35) [2]	$0.05 < p \mid r = 3 < 0.1$
> 35	1 (0.70)	572 (5.88) [2]	
	141	9 711	

2 = total number of mothers 1965–70: 9 711

Table VI *Duration of first and second-stage pains (total labour pains)*

Figures within parentheses denote percentages

Hours	Material	Control*	χ^2-test
< 12	118 (83.68)	406 (81.20) [8]	
12–24	20 (14.18)	74 (14.80) [8]	$\chi^2 = 0.741$
> 24	1 (0.70)	8 (1.60) [8]	$0.6 < p \mid r = \; < 0.7$
Cesarian section without pains	2 (1.41)	12 (2.40) [8]	
	141	500	

8 = 500 consecutive cases

Table VII *Duration of second-stage pains*

Figures within parentheses denote percentages

Minutes	Material	Control*	χ^2-test
> 15	98 (69.50)	289 (57.80) [8]	$\chi^2 = 5.817$
< 15	43 (30.50)	211 (42.20) [8]	$0.01 < p \mid r = 1 < 0.025$
	141	500	

8 = 500 consecutive cases

(g) *Foetal presentations.* There was a tendency to a lower frequency of breech presentations in the series than in the control group, whereas all the other presentations seem to occur with equal frequency (Table XI).

(h) *Number of right and left dislocations in the LOA and ROA-presentations.* The material shows dislocation to the right in two thirds and to the left in one third of the infants born in LOA presentation. This difference is significant ($p = 0.05$). Correspondingly there was dis-

Table VIII *Duration of second-stage pains > 15, in primi- and multipara in OA-presentations*

Figures within parentheses denote percentages

	Material	Control*	χ^2-test
Primipara			
> 15	61 (87.14)	14 (90.68)	$\chi^2 = 0.4036$
< 15	9 (12.86)	22 (9.32) [9]	$0.5 < p \mid r = 1 < 0.6$
	70	236	
Multipara			
> 15	26 (47.27)	82 (31.06) [9]	$\chi^2 = 4.643$
< 15	29 (52.73)	182 (68.94) [9]	$0.025 < p \mid r = 1 < 0.05$
	55	264	

9 = 500 consecutive OA-presentations.

location to the left in two thirds and to the right in one third of the infants born in ROA-presentation. Possibly because of the small number of cases this difference is not significant (Table XII).

The spontaneous course and the results following reduction according to the method of Metzenbaum (Fig. 13)

In 16 cases of septum dislocation reduction was not performed. At the follow-up examination, on an average 3 months later, spontaneous reduction of the dislocation was not observed in any case. In 14 cases the nose was straight while in one case the same deviation of the apex was found as that after labour. There appears to be a pronounced tendency for the cartilaginous part of the

Table IX. *Complications during labour*

Figures within parentheses denotes percentages

Complication	Material	Confidence limits (p 0.05)	Control*
Cervical rupture	0	(0– 6)	33 (0.46) [1]
Vaginal rupture	4 (2.83)	(0.8–7.1)	117 (1.64) [1]
Perineal rupture	12 (8.51)	(4.5–14.5)	142 (4.16–5.66) [6]
Precipitate delivery	0	(0–2.6)	11 (0.24) [7]
Abruptio placentae	0	(0–2.6)	145 (03) [1]
Rupture of the bag of waters > 6 h before labour	6 (4.25)	(1.6–9.1)	375 (5.26) [1]
Funiculus feti circumflectens	39 (27.65)	(20.7–36.0)	1 518 (20.91) [5]
Imminent, fetal hypoxia	10 (7.09)	(3.5–12.8)	555 (7.64) [5]
Artificial delivery	18 (12.76)	(7.8–19.5)	1 571 (16.18) [4]

1 = Total number of mothers 1965–69: 7 124 4 = total number of living infants 1965–70: 9 707 5 = total number of infants 1965–69: 7 257 6 = total number of mothers 1965–67: 2910 7 = total number of mothers 1965–68: 4 487

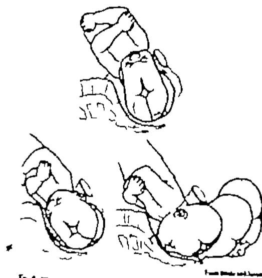


Fig 9 The movements of the foetal head during internal rotation (Steele & Javert, 1942).

Possible nasal trauma during labour

From the moment when labour pains start and the descent is commenced the foetal head, and with that the projecting nose, is affected by innumerable trauma of varying directions and strength (Fig 7). This continues during the further descent of the foetal head (Fig. 8). When the internal rotation commences, the foetal head including the nose, is affected by a well defined trauma, the direction of which is decided only by the foetal presentation in question (Fig. 9)



Fig 10 The occurrence of dislocation of the septal cartilage during rotation in Left Occiput Anterior (LOA) presentation.

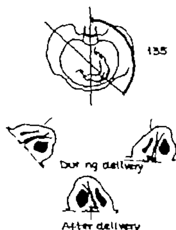


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As OA-presentation comprises 89 % of all foetal presentations, and as the course of labour is best known in this presentation we chose to analyse this part of the series with regard to the possible connection between the direction of the septum dislocation and the foetal presentation.

In the LOA-presentation the internal rotation of the foetal head occurs, observed from the pelvic inlet, in a clockwise direction. The left nasal ala is thus pressed towards the anterior portion of the septal cartilage and then towards the right cheek. It is presumed that the inferior edge of the septum during this rotation is dislocated into the right side of the nose and that the outer nose swings to the left after labour (Fig. 10). Correspondingly the internal rotation in the

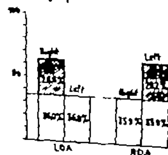


Fig 12 An analysis of the distribution of the direction of dislocations in LOA and ROA presentations in the material.

other on direct vertical pressure (Fig 4 *b*) This procedure is very easy to carry out and should be used in the routine examination of the new born

(*a*) The complications and procedures during pregnancy under study occurred with the same frequency in the series as in the control group although one would expect such factors to cause major trauma to the foetal nose

(*b*) The infants of primipara were affected significantly more frequently This agrees with the observations of Steiner (14)

(*c*) It has not been possible to demonstrate a definite predominance of younger mothers in the material within the age groups chosen.

(*d e*) The total length of the first and second stages of labour does not appear to influence the occurrence of dislocation. However a duration of second stage pains of 15 min or more was more common in the material. It is a wellknown fact that the duration of the second stage is often greater in primiparae than in multiparae. There fore the longer mean duration could be ascribed to the predominance of primiparae However the

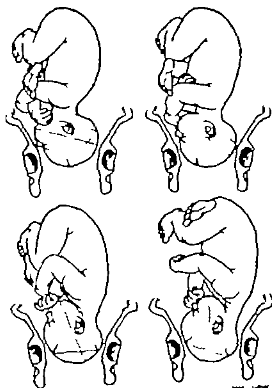


Fig 7 The figure shows the initial movements of the foetal head in the pelvic inlet, necessary for delivery in OA presentation (Rydberg, 1954).

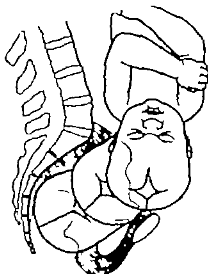


Fig 8 Further descent into the pelvis until engagement (Steele & Javert, 1942).

study demonstrates that the multiparae in the material were responsible for the longer duration of second stage.

(*f*) Labours during which a greater trauma to the foetus might be expected were not more frequent. A significantly lower frequency of moulding was the only factor which differed. This appears to demonstrate that lack of moulding presumably adds to nasal trauma during labour

There was no significantly higher frequency of heavy newborn infants.

(*g*) The different foetal presentations appear with equal frequency in the series and the control group with the exception of Breech-presentation, which was less common in the affected cases. The difference is not significant

(*h*) The fact shown in this study that the inferior edge of the cartilage was dislocated into the right side of the nose in 55% of all cases and in nearly two thirds of the cases delivered in LOA presentation is in contrast to previous reports. According to Briant (1) and Steiner (14) dislocation of the inferior edge to the left should be expected in 70–85% of the cases and should mainly appear after labour in LOA-presentation. Correspondingly dislocation to the left appeared in nearly two thirds of the cases delivered in ROA-presentation in the present study while according to Steiner (14) the dislocation should appear to the right in these cases.

A thorough analysis of the course of labour is therefore of importance.

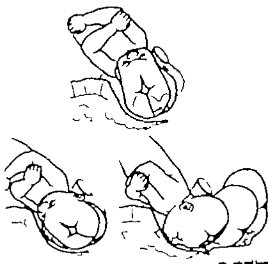


Fig 9 The movements of the foetal head during internal rotation (Steele & Javert, 1942).

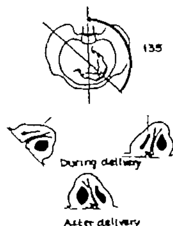


Fig 11 The occurrence of dislocation of the septal cartilage during rotation in Right Occiput Anterior (ROA) presentation.

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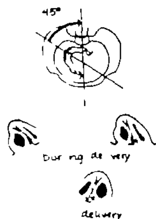


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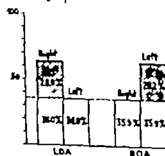


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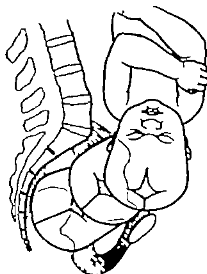


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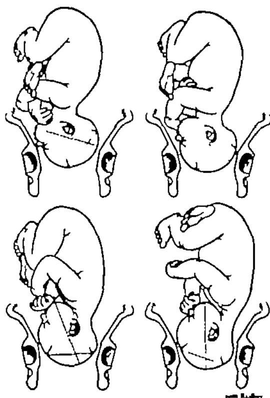


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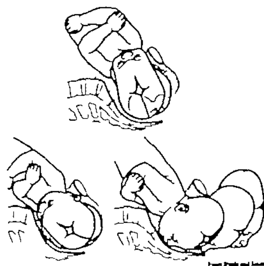


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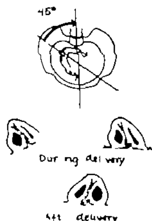


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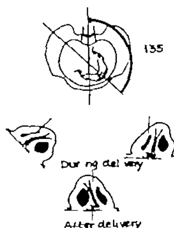


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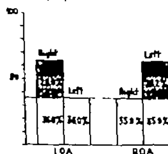


Fig. 12 An analysis of the distribution of the direction of dislocations in LOA and ROA presentations in the material.

Number of pat ents

Treated group 13 — 11 rema nea straight (13 examined) (54 6–98 1 %) p 0,05

Control group 16 — 0 became stra ght spontaneously (15 examined) (0 21 8 %) p 0,05

Fig. 13 The results following reduction ad modum Metzenbaum 14 Sept. 1969 31 March 1970.

ROA presentation will occur as observed from the pelvic inlet, in an anticlockwise direction. The right nasal ala is thus pressed towards the septal cartilage and then towards the left cheek. It is presumed that during this the septum is dislocated into the left side of the nose and that the outer nose swings to the right after birth (Fig. 11)

One would presume that the internal rotation was the cause of the dislocation in the two thirds of the cases in which it takes place to the side expected according to the direction of the internal rotation. However two infants with dislocation were delivered by Caesarean section, before labour started, which shows that septum dislocation can take place during pregnancy Thus in these cases the internal rotation has no influence on the direction of the dislocation. It is probable that dislocation can also be caused by all the varying trauma other than the internal rotation that affect the foetal nose during labour Thus in these cases the internal rotation has no influence on the direction. If the figures from Table XII are now considered in the manner shown in Fig. 12 it is seen that the cases of dislocation distributed according to LOA and ROA-presentation partly occur in two identical groups, within which an equal number of dislocations to the right and left is found. Partly in two equal groups, the character of which is such that they only include cases of dislocation in which the dislocation occurs to the expected side taking into account the direction of the internal rotation

Cases of dislocations occurring during pregnancy and the early stages of labour are caused by trauma from varying directions and may thus statistically be equally represented between the right and left. These cases can explain the two identical groups.

The trauma of the internal rotation has only one well defined direction and is the only one during pregnancy and labour the direction of which is determined by the presentation alone This can explain the remaining two equal groups consisting of one third of the cases, the direction of which is that to be expected from the foetal presentation (Figs. 10 11)

The influence of the length of rotation on the distribution of the dislocations in LOA and ROA

According to Ingerslev (5) the head of the foetus in the OA presentation is in the first oblique diameter (right diameter) on entering the pelvis in two thirds of the cases. This diameter is shown in Figs. 10 and 11 This will result in a rotation of 45 degrees for the two thirds of the foetuses born in the LOA-presentation, while two thirds of the foetuses born in the ROA-presentation rotate 135 degrees. One would immediately assume that a longer rotation would give a greater risk of trauma and therefore a greater frequency of dislocations to the left in the ROA-presentation and a lower frequency of dislocations to the right in LOA presentation would be expected. However the material shows that the frequency is equal (Table XII) Thus the length of rotation is of no importance and the theory of Steiner (14) could not be confirmed

(f) It has been demonstrated that septum dislocations do not reduce spontaneously in contrast to twisting of the outer nose

In a number of cases of deviation of the nasal septum, complications arise such as nasal stenosis and an increased tendency to infections of the upper and lower respiratory tract As reduction probably does not result in additional trauma of any importance this should be carried out and preferably within the first week of life The results following reduction according to the method of Metzenbaum are good, and it is easily performed under local anaesthesia This is in agreement with the literature (6, 8, 9 11) But only continued study of the treated and untreated cases during growth can give the answer to the basic question Should a dislocation of the nasal septal cartilage always be reduced in order to ensure normal physiological function of the nose?

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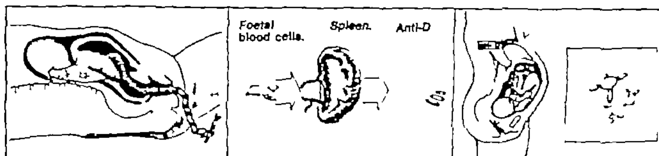
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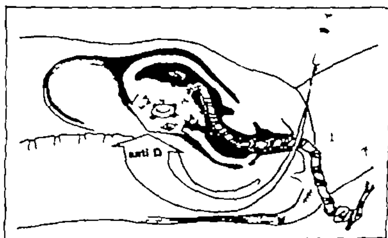
Prevention of Rh-immunization



Schematic illustration of how a small amount of blood crosses over from the foetus to the mother during parturition.

Foetal Rh-positive blood cells elicit the formation of antibodies in the Rh-negative mother

Antibodies pass over from the Rh-immunized mother to the foetus



Prophylactic administration of Gammaglobulin anti-D to the mother within 72 hrs. after delivery in order to prevent Rh-Immunization.

Gammaglobulin anti-D Kabi

without mercury Storage refrigerator Shelf-life 3 years.
Pack 250µg ampoules comb package



Acta Obstet Gynec Scand 51 17–24 1972

THE EFFECT OF HYPOXIA ON THE CATECHOLAMINE CONTENT OF HUMAN FETAL ABDOMINAL PARAGANGLIA AND ADRENAL MEDULLA

Antti Hervonen and Olli Korkala

From the Department of Anatomy (Head: Prof. O. Eränkö), University of Helsinki, Helsinki, Finland

Abstract. The responses of human fetal catecholamine storing tissues, the paraganglia and adrenal medulla to severe oxygen deficiency were tested by using perfusion techniques. The changes in catecholamine content of the target organs were registered by demonstrating the formaldehyde induced fluorescence exhibited by catecholamines. A definite decrease in the intensity of the catecholamine specific fluorescence was observed regularly in the paraganglia after 10–15 min hypoxia. Only very slight changes occurred occasionally in the corresponding adrenal medullae. The role of the target organs and the catecholamine release during oxygen deficiency are discussed.

Several modifications of perfusion methods for human fetuses hav been widely used in studies on the steroid metabolism of the fetoplacental unit. The perfusion of previable fetuses also provides the opportunity to imitate the common pathophysiological disorder fetal asphyxia.

The role of catecholamines in regulation of fetoplacental circulatory dynamics is obviously important. Ehinger et al. (16) noticed that noradrenaline produced a contractile response in the umbilical vein and the ductus venosus also seemed t receiv an adrenergic innerv tion. Aronson et al (2) found a rich adrenergic nerve plexus in the human fetal ductus arteriosus and contractile response was produced by noradrenaline and adrenaline.

Brundin (6) was the first to find a release of catecholamines from the fetal main para-aortic body of the rabbit. Severe asphyxia elicited a marked loss of catecholamine from the para aortic bodies. A direct ffect on paraganglionic ells was suggested. Comline (7 10) studied the dev lopment of secretory responses of fetal calf adrenal medulla. He found a discharge of catecholamine from the fetus after hypoxia, and the effect on chromaffin cells was assumed to be the direct result of chemostimulation. The author also pointed out that the paraganglionic tissue, which contains large amounts of noradrenaline, might reinforce the discharge from adrenal medulla of the fetus.

The possibility of functional innervation of the paraganglia has been widely debated. Although light microscopic data for the innervation is available (20, 24 18, 19) it seems probable that the paraganglia lack functional secretory innervation (6, 12, 13 19).

The widespread distribution and variable anatomy of human fetal adrenal medulla and paraganglia (11) make it technically impossible to obtain satisfactory tissue samples for quantitation of changes in catecholamine content of the paraganglia or adrenal medulla. Instead, qualitative estimations of noradrenaline and adrenaline contents of the paraganglia as well as comparisons between the catecholamine contents of paraganglia and adrenal medulla of the human fetus, have been performed by several investigators (22, 11). Semiquantitative estimation of changes in the catecholamine-specific formaldehyde induced fluorescence (FIF) was therefore the only useful method for evaluation of the catecholamine content of fetal chromaffin tissue. Microfluorimetric studies in the same field are in progress in our laboratory.

The present study was undertaken to elucidate the functional role of fetal paraganglia and adrenal medulla.

MATERIAL AND METHODS

The perfusion apparatus as constructed following the principles presented by Westin et al. (25) and Diczfalusy

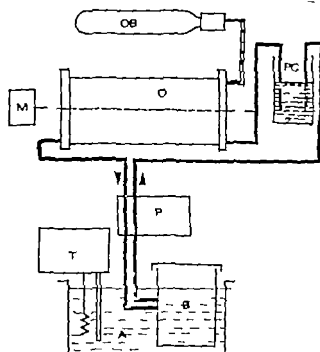


Fig 1 The perfusion apparatus constructed for human fetuses. The extracorporeal circulation starts from the perfusate container (*PC*) from which the flow i directed to the oxygenator (*O*) rotated by a motor (*M*). Oxygen is flowing through the air space of the oxygenator with adjustable rate from the oxygen cylinder (*OB*). After oxygenation the perfusate is pumped (*P*) to the fetus box (*B*) where the flow is connected to the umbilical vessels. The outflow again is elevated back to the per fusate container if not collected for chemical analyses. The artificial amnionic bath (*A*) is warmed with automatic thermostatmixer (*T*) which maintains the temperature within limits of ±1°C.

(15). The apparatus consisted of two main parts (Fig. 1): perfusion chamber and the ygenator

The pressure pe fusion chambe was essential to prevent the increase of total fluid volume inside the fetus (Westin, 25). The chamber was not used when older fetuses were processed, since the circulation could be maintained for the relatively short experimental period (max. 15 min) w thout difficulties. Only minor differences between the input volume and output volume were measured. Fetuses younger tha 16 weeks were perfused under volume control in the pressure chamber

The umbilical artery of the fetus was connected to a thin vinyl catheter (Intrachat®) which served as the perfusion input. Another catheter was placed in the umbilical vein and the tubes were fixed with silk ligatures. The fetus was placed in the pressure chamber which was closed and rapidly filled completely with isotonic saline with 2% glucose added. The perfusion circuit was connected to the fetus and, in our hands, the circulation started usually without further delay. Coagulation of the perfusate sometime occurred in the output catheter. This was relieved by suction with a yringe.

The circulation thus started ~3 min after separation of the fetomaternal contact. The closed system provided a continuous and stable circulation, because the volume of the fetus could not change, and the input volume must therefore balance the outflow

The oxygenator was constructed of sheets of perspex and it consisted of parts which could easily be separated for cleaning. The oxygenator rotated at 60 r.p.m. The perfusate flowed continuously to the oxygenator by gravity feed from a contained placed at a higher level. The effective area of the oxygenator for surface contact of oxygen with the perfusate was about 3 000 cm². The continuous flow of oxygen through the chamber could be regulated with a flow meter. Foam formation was avoided by adjusting the rotation rate at 60 r.p.m. and oxygen flow under 10 l/min. The usual perfusion pressure was 50 cm/H_2O. The oxygenator chamber was also connected to a manual rubber pump for momentary pressure elevations needed when the circulation as accidentally disturbed.

The perfusate consisted of 20% erythrocyte suspension in Krebs-Ringer-glucose buffer. The possible incompatibility of blood groups was not taken into consideration because of the relatively short perfusion time and because the fetal blood volume was rapidly replaced by the perfusat. Agglutination did not disturb the procedure.

The fetuses were obtained from legal interruptions of pregnancy performed by laparotomy. The Crown-Rump-lengths are listed in Table I. Only technically successful perfusions were accepted. After 15 min perfusion the specimens from the paraganglia and adrenals were quickly prepared for freeze drying.

A tissue block containing the main paraganglia was removed first, and frozen in isopenthane or propane

Table I *Intensity of FIF in perfused human fetal paraaortic bodies and adrenal medullary tissue*

Maximal intensity in control material

CR-length	PAB	AM
Control perfusion		
7.1		
8.5		
13.9		
15.3		
Hypoxia perfusion		
5.1		
8.6		
10.1		
10.5		
10.9		
15.5		
Anoxia perfusion		
8.8		
8.8	( )	
9.8	( )	
11.5		
11.7		
13.3		
13.6		

cooled with liquid nitrogen. Adrenal slices of 1 mm were then prepared and frozen. The drying procedure was performed in constant conditions: (*a*) drying temperature 37°C, (*b*) drying vacuum 10^{-4} torr, (*c*) drying time 48 hours. Large amounts of phosphorous pentoxide were used as an effective chemical water trap (23). The exposure to formaldehyde vapour was performed following the general rules presented by Eränkö (17). The usual combination of technical values was: (*a*) Exposure temperature 80°C. (*b*) Humidity of the paraformaldehyde powder 60–70% (*c*) Exposure time 30 min.

The specimens were embedded in epon-araldite mixture and 2 μm thick sections were cut with an LKB pyramitome. The intensity of the formaldehyde induced fluorescence was estimated from the main paraaortic bodies and adrenomedullary collections of chromaffin cells. The fluorescent microscopy was performed with Wild monocular microscope equipped with mercury lamp HBO 200 (Osram).

RESULTS

The control material consisted of immediately freeze-dried material described previously (19). The intensity of the formaldehyde induced fluorescence (FIF) increased in both paraganglia and adrenomedullary cells until the fourth month, when it reached a maximal level for subjective evaluation of the intensity. Therefore only fetuses older than 12 weeks were used.

The perfusion controls were processed as follows. The maximal oxygenation capacity was used and the fetuses were perfused for 10–15 min and processed further as described. The purpose of this control group was to eliminate the artefacts caused by the procedure itself and to evaluate the changes in the catecholamine content of the cells caused by factors other than oxygen deficiency.

The results of the estimations are given in Table 1. To summarize, no changes could be found in the FIF of the perfusion control material, the brightly yellow FIF found in the native controls was also present in perfusion controls. In the toluidin blue stainings made after the estimations of FIF occasional extravasations of perfusate were found. A slight widening of capillary sinusoids of adrenal medulla and paraganglia was also evident.

Hypoxia

The oxygen saturation of the perfusate was lowered by ventilating the oxygenator with air and diminishing the rotation rate of the apparatus.

The decrease of oxygen saturation of the perfusate induced a clearly recognizable decrease in the fluorescence intensity of paraganglia. These changes were noted in the adrenals of some fetuses, but not in all (see Table I).

Severe hypoxia (anoxia) was caused by perfusing the fetuses with non-oxygenated perfusate, by-passing the oxygenator. The decrease in the intensity of FIF was already evident after 10 min perfusion in both localisations of catecholamine-storing cells. In the main para-aortic bodies, almost complete disappearance of FIF was found in some areas. Cells with moderate FIF were left, scattered throughout the body, but the great majority of the paraganglia showed only weak green FIF. After 10 min severe hypoxia, the decrease was already evident, but the bright yellow FIF was still dominant.

The adrenomedullary cells also lost their intense yellow FIF, but the change was not so marked as in the paraganglia of the same fetus. It was also more difficult to evaluate, because of the widespread distribution of the groups of medullary cells. Hence, the possibility of complete loss of fluorescence in some catecholamine-storing cells could not be excluded. However, the general change was clear: a decrease of fluorescence intensity, but not so marked as in the paraganglia.

Because of the restricted age range of the fetuses the possible development of the response to hypoxia remained unclear.

The distribution of FIF in the para-aortic body of hypoxia-perfused material followed certain rules. After a mild, 10 min hypoxia, the loss of FIF was most marked in cells on the capillary sinusoids. The medullary cells without direct contact with the capillary wall did not show any changes in the intensity of FIF. After severe hypoxia, no such differences depending on the situation of the cells in relation to capillary sinusoids were found: all cells had lost their yellow FIF, and only traces of greenish fluorescence were found.

DISCUSSION

It is well known that certain pathophysiologic conditions during the fetal life, such as asphyxia, stimulate the release of catecholamine from the adrenal gland. Comline et al. (7–10) exhaustively studied the responses of the adrenals of fetal and

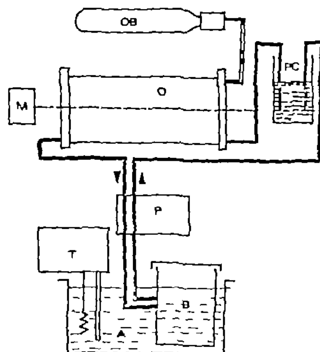


Fig 1 The perfusion apparatus constructed for human fetuses. The extracorporeal circulation starts from the perfusate container (*PC*) from which the flow is directed to the oxygenator (*O*) rotated by a motor (*M*). Oxygen is flowing through the air space of the oxygenator with adjustable rate from the oxygen cylinder (*OB*). After oxygenation the perfusate is pumped (*P*) to the fetus box (*B*) where the flow is connected to the umbilical vessels. The outflow gain is elevated back to the per fusate container if not collected for chemical analyses. The artificial amniotic bath (*A*) is warmed with automatic thermostatmixer (*T*) which maintains the temperature w thin limits of +1°C.

(15). The pparatus consisted of two main parts (Fig. 1). *perfusion chamber and the oxygenator*

The pressure perfusion chamber was essential to prevent the increase of total fluid volume inside the fetus (Westin, 25). The chamber was not used when older fetuses were processed, since the circulation could be maintained for the relatively short e perimental period (max. 15 min) without difficulties. Only minor differences bet een the input volume and output lume wer measured. Fetuses younger than 16 weeks were perfused under v lume control in the pressure chamber

The umbilical artery of the fetus was connected to thin vinyl catheter (*Intrachat®) which served as the* perfusion input. Another catheter was placed in the umbilical vein and the tubes were fixed with silk ligatures. The fetus was placed in the pressure chamber which was closed and rapidly filled completely with isotonic saline with % glucose added. Th perfusion circuit was connected to the fetus and, in our hands, the circulation started usually without furthe delay Coagulation of the perfusate sometime occurred in the output cathet r This was relieved by suction with syringe

The circulation thus started ~3 min after separation of the fetomaternal contact. The closed system provided a continuous and stable circulation, because the volume of the fetus could not change, and the input volume must therefore balance the outflow

The oxygenator was constructed of sheets of perspex and it consisted of parts which could easily be separated for cleaning. The oxygenator rotated at 60 r.p.m. The perfusate flowed continuously to the oxygenator by gravity feed from a contained placed at a higher level. The effecti e area of the oxygenator for surface contact of oxygen with the perfusate was about 3 000 cm² The continuous flow of oxygen through the chamber could be regulated with a flow meter Foam formation was avoided by adjusting the rotation rate at 60 r.p.m. and oxygen flow under 10 l/min. The usual perfusion pressure was 50 cm/H_2O The oxygenator chamber was also connected to a manual rubber pump for momentary pressure elevations needed when the circulation as accidentally disturbed.

The perfusate consisted of 20 erythrocyte suspension in Krebs-Ringer-glucose buffer The possible incompatibility of blood groups was not taken into consideration because of the relatively short perfusion time and because the fetal blood volume was rapidly replaced by the perfusate Agglutination did not disturb the procedure.

The f tuses were obtained from legal interruptions of pregnancy performed by laparotomy The Crown-Rump lengths are listed in Table I. Only technically successful perfusions were accepted. After 15 mi perfusion the specimens from the paraganglia and adrenals were quickly prepared for freeze drying.

A tissue block containing the main paraganglia was removed first, and frozen in isopenthane or propane

Table I. *Intensity of FIF in perfused human fetal para-aortic bodies and adrenal medullary tissue*

M ximal intensity in control material.

CR-length	PAB	AM
Control perfusion		
7 1		
8.5		
13 9		
15.3		
Hypoxia perfusion		
5 1		
8.6		
10.1		
10 5		
10.9		
15.5		
Anoxia perfusion		
8 3		
8.8	( )	
9.8	( )	
11.5		
11 7		
13.3		
13.6		

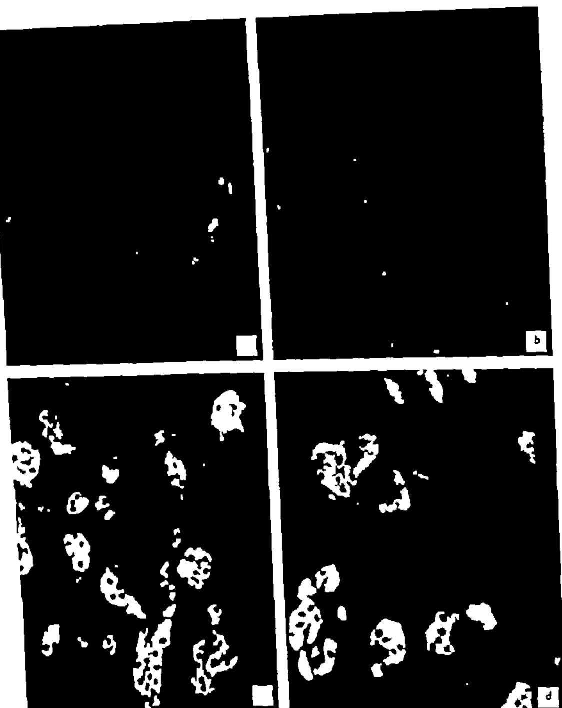


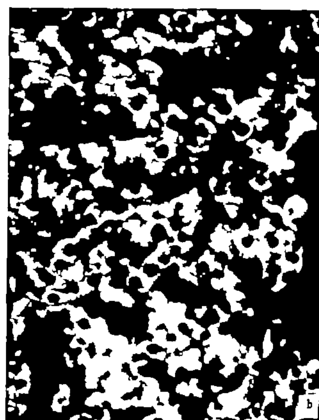
Fig. 3 (*a*) The main para-aortic body after hypoxic perfusion for 1 min. The organ is partially nonfluorescent, but no brightly yellow FIF could be found in the cells; slight diffusion artefacts are visible × 375.

(*b*) The main para-aortic body after 1 min perfusion with anoxic perfusate. The paraganglionic cells showed only weak greenish FIF. The intensity of FIF was unusual in all cells. × 375

(*c*) The adrenomedullary CA-storing cells in 16-week old fetus. The cells exhibited bright yellow FIF × 280.

(*d*) The adrenomedullary cells after 15 min anoxia. Only slight decrease of FIF could be found. Slight diffusion artefacts could also be found × 280.





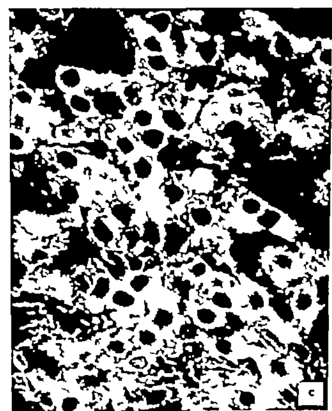




Fig 2 ( ). The main para-aortic body f 14-week-old fetus. Normal control specimen hich has been freeze dried mmediat ly after delivery All cells exhibit an even brightly y llow FIF 10

(*b*) N rmal control An rea f thi main para-aortic body f a 16-week-old fetus. The c lumns f CA-storing cells are separated by wide capillary sinusoids. 370.

( ) The perfusion control The pecimen has been perfused w th maximally xygenated perfusate for 15 min. The intensity f FIF exhibited by the paraganglionic cells remained nchanged. Only sl ght diffusion rtefacts could be found. 745

(*d*) The hypoxi perfusion. The pecimen has been perfused for 10 mi w th nly partially oxygen saturated fluid. The pericapillary nonfluorescing areas (arrow) are wider and weekly fluorescing cellprofiles could be observed. Also brightly y llow fluo escine cells were present. 370.

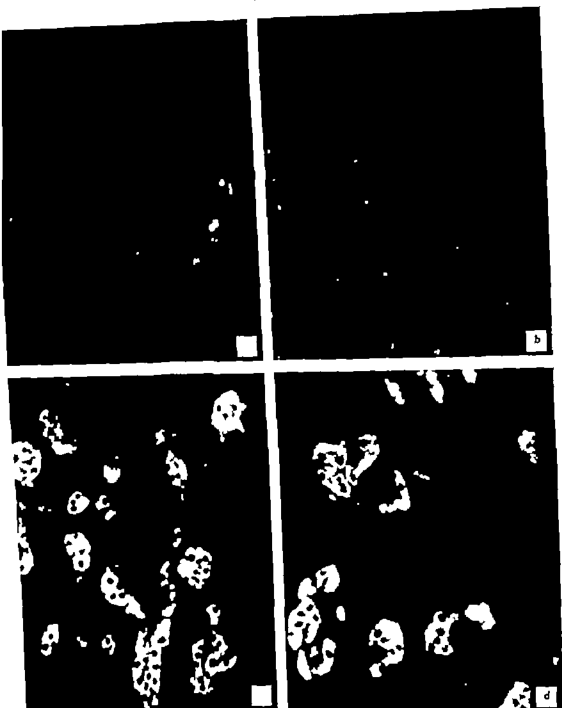


Fig. 3 (*a*) The main para-aortic body after hypoxic perfusion for 15 min. The organ is partially nonfluorescent, but also brightly yellow FIF could be found in the cells, slight diffusion artefacts are visible. ×375.

(*b*) The main para-aortic body after 15 min perfusion with anoxic perfusate. The paraganglionic cells showed only weak greenish FIF. The intensity of FIF was minimal in all cells. ×375

(*c*) The adrenomedullary CA-storing cells in 16-week old fetus. The cells exhibited bright yellow FIF ×280.

(*d*) The adrenomedullary cells after 15 min anoxia. Only slight decrease of FIF could be found. Slight diffusion artefacts could also be found. ×280

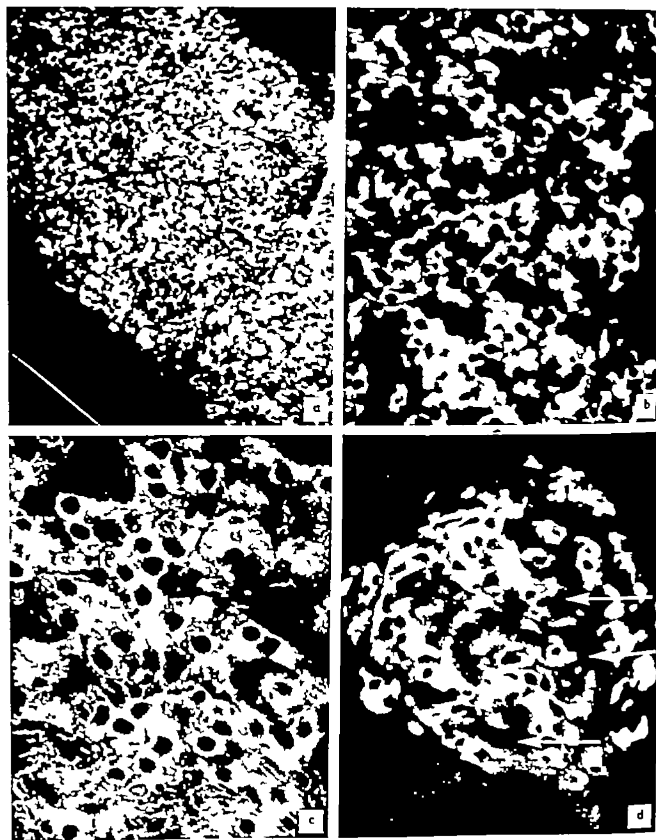


Fig 2 ( ) The main para-aortic body f a 14- eek-old fetus Normal control specimen which has been freeze dried immediately after delivery All cell exhibit an even brightly y flow FIF 10

(*b*) Normal control. An area of th main para-aorti body f 16- eek-old fetus The columns of CA-storing cell are separated by wide capillary sinusoids 370.

( ) The perfusion co trol The specimen has been perfused w th maximally ygenated perfusate for 15 min. The intensity of FIF exh bited by the paraga glionic cells remained unchanged. Only slight diff uo artef cts could be found. 745

(*d*) The hypoxia perfusion. The specimen has been perfused for 10 min with only partially oxygen saturated fluid. The pericapillary nonfluoresci g areas (rrows) are wider and weekly fluorescing cellprofiles could be observed. Also brightly yellow fluorescing cells w re present. × 370.

several fetal tissues is evident (14) and this effect proved to be of importance in adverse environmental conditions for the newborn lamb (1). A prolonged asphyxial situation could produce continuous release of catecholamines from the paraganglia and the calorigenic effect of noradrenaline may help to balance the nutritional and environmental disorders.

The adrenal medulla did not respond to hypoxia as clearly as the paraganglia. On the other hand, morphologically mature nervous supply is present in the medulla of the fetuses studied (19). The effect of hypoxia on medullary chromaffin cells could thus be mediated by the preganglionic innervation. The results were in agreement with the findings of Brundin (6), who did not find any decrease in catecholamine content of rabbit adrenal medulla after hypoxia.—Difference in the catecholamine-discharge mechanisms of adrenomedullary and paraganglionic cells existed. It is not possible to predict which alternative catecholamine secretion mechanisms would be dominant at term, but during the second trimester of pregnancy the direct humoral regulation of the paraganglia seems to be dominant.

Electron microscopic studies on the changes of the fine structure of the catecholamine storing cells produced by the hypoxia and other chemical factors working in the fetal asphyxia are in progress.

ACKNOWLEDGEMENTS

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newborn calf It was evident that noradrenaline was the dominating amine discharged from the sheep and calf chromaffin tissue during hypoxia. The possible contribution of paraganglia to the discharge remained unclear and no evidence for secretory response from paraganglia was presented.

Brundin (6) showed that rabbit para-aortic body released considerable amounts of noradrenaline in hypoxia. He suggested that the paraganglia were of importance in fetal asphyxia. Both authors came to the conclusion that the effect of hypoxia on fetal chromaffin tissue is direct, and not mediated by the preganglionic innervation. Comline et al. (10) suggested that the mechanism for release of noradrenaline from the fetal cells is different from that in the adult.

Considering the results mentioned above it was not surprising to note the decrease of the formaldehyde induced fluorescence after hypoxia. The observation of FIF-intensity by eye is not an accurate method for detecting minor changes in catecholamine content of tissues. Jonsson (21) showed that, after the quenching of the fluorescence caused by high concentrations of nor adrenaline larger changes in amine concentration may also escape detection Thus lowers the value of the perfusion controls, which showed unchanged intensity of the fluorescence. The real concentration of catecholamines after the control perfusion could be evaluated by microfluori metric techniques. However the present perfusion technique makes long fetal survival periods possible according to Westin et al. (25) who maintained the circulation for 5–12 hours. In the present work, only a relatively short period of survival was required (15 min) and it is probable that only minor changes in the catecholamine stores occurred during this time.

The marked decrease of the fluorescence intensity evidently indicated discharge of catecholamines from the paraganglia. This effect is probably caused by direct stimulation of the para ganglionic cells by the decreased oxygen concentration because no functional nerve endings could be demonstrated during this period of fetal life in the paraganglia (19) Hence the mechanism of catecholamine release was evidently not un like the discharge from rabbit paraganglia (6) Anoxia of 10 min duration already caused a visible change in the fluorescence According to Jonsson (21) It is thus probable that large amounts of catecholamines must have been discharged from the tissue during the short but severe oxygen deficiency Brundin (6) obtained similar results after longer exposure of the animals to lowered oxygen concentration. The rapid response found may indicate that a proportion of the paraganglionic catecholamines is loosely bound and easily released by a sudden drop in oxygen concentration.

The functions of the catecholamines in the fetal organism may be concerned with the control of haemodynamics of the fetoplacental circulation. The blood flow in the umbilical cord, umbilical vein and ductus venosus of human fetuses is probably controlled by the adrenergic sphincter mechanism (16) Injected noradrenaline produced a contractile response in these vessels. Born et al (4) have found that infusion of either adrenaline or noradrenaline causes contractions of the ductus arteriosus of the lamb fetus and according to Comline et al. (7) enough catecholamine is present in the circulation during oxygen deficiency to cause the contraction Recently Aronson et al. (2) confirmed these effects of adrenaline and nor adrenaline on the ductus arteriosus of human fetuses. These authors suggested that nor adrenaline plays a functional role in the closure of ductus arteriosus at term. It is evident that noradrenaline in particular if released in the fetal circulation, would produce some changes in the haemodynamics of the fetoplacental unit. It is well known that a variable degree of hypoxia of the fetus cannot be avoided at delivery Bartels (3) measured very low pO_2 from the umbilical cord blood during delivery The release of catecholamines from the paraganglia of human fetuses might be of importance when the physiologic changes in the fetal circulation occur at term The contraction of the ductus arteriosus and the blood channels of the umbilical cord and ductus venosus are essential features of these haemodynamic changes. Recently Brinkman et al. (5) suggested an active vasoconstriction in the um bilicoplacental circulation during acute hypoxia. According to these authors it was likely that these changes occurred in the umbilical venous circulation and might also explain some of the other cardiopulmonary haemodynamic changes oc curring simultaneously

The calorigenic effect of noradrenaline on

PLASMA OESTRIOL IN PROLONGED PREGNANCY

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Abstract. Plasma oestriol concentrations were studied in 43 women with prolonged pregnancies. In all, 177 studies were made from the 40th to 46th weeks. There was a significant fall in plasma concentration from the 40th to 46th weeks contrasting with the significant rise from the 24th to 42nd weeks of normal pregnancy. There was no correlation between the plasma concentration and urinary excretion of oestriol, as there is in normal pregnancy from the 24th to 42nd weeks, but there was a significant difference in the correlations (at these two stages) pointing to an increased renal clearance of oestriol during prolonged pregnancy. It is postulated that this phenomenon might depend on a disproportionate increase in the production of the conjugate oestriol-16-glucosiduronate, which is more rapidly excreted by the kidneys than oestriol-3-sulphate, and which reflects reduced metabolic activity. Urinary oestriol excretion, however, falls during prolonged pregnancy despite the postulated increased clearance. It is concluded, therefore, that oestriol production, which reflects the function of the feto-placental unit, is, in fact, reduced during prolonged pregnancy. Plasma oestriol studies would appear, therefore, to give a better physiological control of prolonged pregnancy than urinary oestriol studies.

The incidence of prolonged pregnancy reported from larger studies varies between 5 and 12% (3, 18, 29, 35). This variation depends to some extent on differences in the definitions of prolonged pregnancy. In this department, prolonged pregnancy is defined as pregnancy continuing more than 294 days after the first day of the last menstrual period. The calculation presupposes that the menstrual cycle is regular (25–35 days), and that the time of quickening is as expected (34). On the basis of this definition the incidence of prolonged pregnancy in this department is approximately 10%.

There is not agreement in the literature on the treatment of prolonged pregnancy, but several reports have documented increased fetal risk: the perinatal mortality increases, especially in primiparous (22, 32); "fetal distress" (intra-uterine and neonatal asphyxia, abnormal heart sounds, and fetal acidosis) is more common (1, 11, 12); the frequency of birth weights over 4000 g, and of caesarean section, is increased (1). Not all, however, find increased mortality (21), nor increased frequency of acute or chronic fetal hypoxia (24).

If treatment is expectant, patients should be frequently examined. Amnioscopy should be performed, and the urinary excretion of oestriol measured. The former will exclude meconium stained fluid, which is a bad prognostic sign (18), and the latter is of value because positive correlation between reduced oestriol excretion and "fetal distress" has been demonstrated (1, 6, 11, 14, 23).

Cytohormonal studies of vaginal smears (26) or amnioscopy alone (17) do not seem to be adequate controls. Urinary oestriol excretion is, at present, the best measure of the function of the feto-placental unit, and if excretion decreases, pregnancy should be terminated.

Plasma oestriol in prolonged pregnancy has not been much studied. Schwers (30) reports nine values, and Taylor et al. (33) five from the 42nd week or later. Falling plasma concentrations were observed in both studies.

In this present investigation plasma oestriol studies were made in women with prolonged pregnancies from the 40th week onwards. In general, there were no apparent complications during pregnancy and treatment was therefore expectant.

MATERIAL AND METHODS

In all, 177 plasma oestriol determinations were made on samples taken from 48 women between the 40th and 46th

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RESULTS

Table IV gives the arithmetic mean values and standard deviations for oestriol in plasma and in urine from the 40th to 46th weeks. Mean values of oestriol in both plasma and urine tend to decrease as pregnancy proceeds. That there are more plasma than urine determinations reflects the greater facility of obtaining blood samples. Table V shows that the last plasma determination was made within two days of delivery in 18 patients, and within one week of delivery in 44 patients. Several authors have found that the blood oestrogen concentrations do not fall during the first stage of labour from the values of the preceding day (19, 20, 25, 27, 30) and therefore determinations made on women admitted in the first stage (six in all) are included in this study: two of these patients showed falls in plasma oestriol concentration compared with earlier determinations; two had falling concentrations over the last 14 days before delivery; one had a constant concentration over 21 days before delivery and one showed an increased oestriol level by comparison with the previous week. It may be wrong to include this last patient, in whom labour was oxytocin induced, which in some cases has been reported to increase plasma oestriol concentration (19, 20).

Fig. 1 shows the individual plasma oestriol determinations plotted against the day of pregnancy on which the samples were obtained. Nearly all values lie within the normal range for the 40th to 42nd weeks. Also shown on the figure is (a) the regression line for the mean values of plasma oestriol for the 24th to 42nd weeks of normal pregnancy, with the 95% limits for all values ($\log Y_1 = 0.8489 + 0.0317 X$, $s_{b_1} = 0.0025$, $N = 279$, where Y = plasma oestriol concentration (µg/litre) and X = weeks of pregnancy) and (b) the regression line with 95% limits for plasma oestriol in prolonged pregnancy from the 40th to the 46th weeks ($\log Y = 2.9901 - 0.0029 X_2$, $s_{b_2} = 0.0019$, $N = 177$). The slope of the prolonged

Table IV. *Oestriol concentrations in plasma and in urine of 48 patients with prolonged pregnancy from the 40th to 46th week of pregnancy*

Mean values ± standard deviations (m ± S.D.). N: No. of analyses

	Concentration in plasma (µg/l)		Excretion in urine (mg/24 h)	
Week	m ± S.D.	N	m ± S.D.	N
40	148 ± 63	20	26.3 ± 9.9	21
41	165 ± 77	47	25.2 ± 5.5	39
42	156 ± 79	51	24.5 ± 7.9	48
43	148 ± 69	40	25.8 ± 9.4	34
44	148 ± 111	11	23.7 ± 8.3	10
45	151 ± 80	5	20.0 ± 7.9	4
46	142	3	22.7	2
Total		177		158

Table V. *Distribution of patients according to the interval between the last plasma oestriol analysis and delivery*

Interval days	No. of Patients
>0–≤1	18
>1–≤3	18
>3–≤7	8
>7	4
Total	48

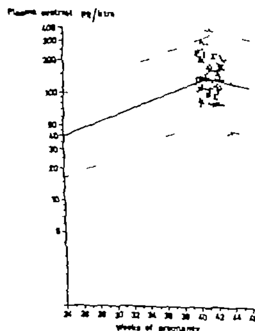


Fig. 1. The regression line and 95% limits for the mean values of 177 plasma oestriol analyses in prolonged pregnancy (40th to 46th weeks). The regression line and 95% limits for plasma oestriol in normal pregnancy (24th to 42nd weeks) are appended. Logarithmic ordinate.

Table I *Distribution of 48 patients with prolonged pregnancy according to number of pregnancies and parity*

No.	Pregnancies (No. of pts.)	Parity (No of pts.)
I	25	29
II	11	10
III	6	4
≥IV	6	5
Total	48	48

Table III *Products of 48 prolonged pregnancies. Distribution of babies according to birth weight*

Birth weight (g)	No. of babies
>2 000–≤2 500	1
>2 500–≤3 000	3
>3 000–≤3 500	17
>3 500–≤4 000	18
>4 000–≤4 500	9
Total	48

week of pregnancy Weekly blood samples for plasma oestriol analysis were taken from all women attending the antenatal clinic from the time they entered the 40th week of pregnancy The studies were made over a five month period in the hospital s ante-natal clinic. Analyses were nearly always in duplicate. At the same time 158 urinary oestriol analyses were undertaken on specimens collected in the 24 hours immediately preceding venepuncture for the plasma analyses. The first analyses were made t about the expected date of delivery (40th–41st week) and subsequently at weekly intervals. After the 42nd week, so far far as possible, analyses were made every third r fourth day The average number of analyses made in each patient was 3,8

Four patients did not fulfil exactly the criteria in the above mentioned definition of prolonged pregnancy Their menstrual cycles were irregular but the first fetal movements were felt at the time expected from the calculated date of delivery which is reliable confirmatory sign of this date (28) In ddition, pregnancy could be related to specific act of coitus in one instance and in all the patients the uterine size tallied with the calculated duration of pregnancy In the remaining 44 patients all criteria were satisfied, and all parameters supported the calculation of term.

Women who had taken oral contraceptives in the 4–6 months before their last menstrual period ere excluded, as also were patients with any c mplication f pregnancy particularly decreasing urinary oestriol, which might dictate termination at or before term.

Five patients had some bleeding ea ly in pregnancy which could not be confused with menstrual bleeding. It is of interest that a significant positive correlation has been found between vaginal bleeding in the first 13 eks of pregnancy and prolonged pregnancy (3).

The distribution of patients according t number of pregnancies and parity is shown in Table 1 The average ge of primiparous was 23.. years ± 4.5 years, and of multiparous 27.8 years ± 5.2 years. Labour began spontaneously in 35 patients, and was induced with oxytocin in eight (six patients with large babies, one patient ith hypertension, and one with falling rinary excretion of oestriol). Caesarean section was performed in the remaining five patients for the following indications planned sterilization earlier vaginal peration for genital prolapse failed induction of labour after escape of amniotic fluid 37 hours previously feto-pelvic dispropor tion and abnormal fetal heart sounds associated ith primary inertia of labour

Table II gives the duration f the pregnancies, and Table III the birth weights of the babies. Approximately 20% weighed more than 4 000 g Neonatal asphyxia occurred in only four babies (three with Saling scores f 8, 9 and 6, and one with an Apgar f 5). Saling or Apgar scores w re not recorded in four cases, but the babies were probably not asphyxiated. Seven babies ere described as dysmature (sparse vernix caseosa and subcutaneous fat, wrinkled, dry skin), and 13 as not dysmature The remaining 28 babies re not described ith special reference to dysmaturity but gain, from the records as whole, it is improbable that any ere dysmature

One baby died three days after birth following operation for co-arctation of the aorta.

Forty-three patients were monitored by repeated amnioscopy and measurement of urinary oestriol xcretion. Medical inductions of labour were twice performed because amniotic fluid was sparse and not clear One patient had mild green discolouration f the fluid, but had an uncomplicated birth of normal baby shortly afterwards.

Small placental infarcts (respectively 1 cm and 5 cm in diameter) were seen in two patients

The plasma oestriol analyses ere made in the department laboratory using a gas chromatographic method (9) The mean values and 95% limits betwee the 4th and 42nd eeks of normal pregnancies have been reported earlier (8). Urinary oestriol determinations re made in the Hormone Department, Statens Seruminstitut, Copenhagen, using Frandsen method (13).

Table II *Distribution of 48 patients with prolonged pregnancy according to the duration of pregnancy*

Duration of pregnancy	No. of pts.
>294–≤297	23
>297 301	15
>301 308	4
>308– 315	3
>315– 322	3
Total	48

RESULTS

Table IV gives the arithmetic mean values and standard deviations for oestriol in plasma and in urine from the 40th to 46th weeks. Mean values of oestriol in both plasma and urine tend to decrease as pregnancy proceeds. That there are more plasma than urine determinations reflects the greater facility of obtaining blood samples. Table V shows that the last plasma determination was made within two days of delivery in 18 patients, and within one week of delivery in 44 patients. Several authors have found that the blood oestrogen concentrations do not fall during the first stage of labour from the values of the preceding day (19, 20, 25, 27, 30), and therefore determinations made on women admitted in the first stage (six in all) are included in this study: two of these patients showed falls in plasma oestriol concentration compared with earlier determinations; two had falling concentrations over the last 14 days before delivery; one had a constant concentration over 21 days before delivery and one showed an increased oestriol level by comparison with the previous week. It may be wrong to include this last patient, in whom labour was oxytocin induced, which in some cases has been reported to increase plasma oestriol concentration (19, 20).

Fig. 1 shows the individual plasma oestriol determinations plotted against the day of pregnancy on which the samples were obtained. Nearly all values lie within the normal range for the 40th to 42nd weeks. Also shown on the figure is (*a*) the regression line for the mean values of plasma oestriol for the 24th to 42nd weeks of normal pregnancy with the 95% limits for all values ($\log Y_1 = 0.8489 + 0.0317 X_1$, $s_b = 0.0025$, $N = 279$, where Y = plasma oestriol concentration (µg/litre) and X = weeks of pregnancy) and (*b*) the regression line with 95% limits for plasma oestriol in prolonged pregnancy from the 40th to the 46th weeks ($\log Y_2 = 2.9901 - 0.0029 X_2$, $s = 0.0019$, $N = 177$). The slope of the prolonged

Table IV. *Oestriol concentrations in plasma and in urine of 48 patients with prolonged pregnancy from the 40th to 46th weeks of pregnancy*

Mean values ± standard deviations (m ± S.D.). N = No. of analyses

Week	Concentration in plasma (µg/l) m ± S.D.	N	Excretion in urine (mg/24 h) m ± S.D.	N
40	148 ± 63	20	26.5 ± 9.9	21
41	165 ± 77	47	25.2 ± 5.5	39
42	156 ± 79	51	24.5 ± 7.9	48
43	148 ± 69	40	25.8 ± 9.4	34
44	146 ± 111	11	23.7 ± 8.3	10
45	151 ± 80	5	20.8 ± 7.5	4
46	142	3	22.7	2
Total		177		158

Table V. *Distribution of patients according to the interval between the last plasma oestriol analysis and delivery*

Interval days	No. of Patients
>0–≤1	18
>1–≤3	18
>3–≤7	8
>7	4
Total	48

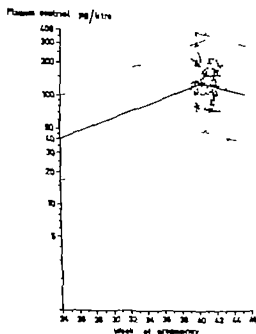


Fig. 1. The regression line and 95% limits for the mean values of 177 plasma oestriol analyses in prolonged pregnancy (40th to 46th weeks). The regression line and 95% limits for plasma oestriol in normal pregnancy (24th to 42nd weeks) are appended. Logarithmic ordinate.

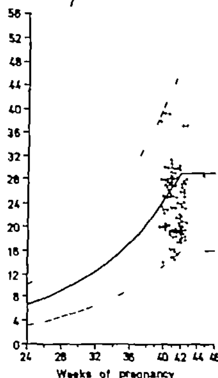


Fig 158 urinary oestriol analyses in prolonged pregnancy (40th to 46th weeks), and curves for the mean values and 95% *limits in normal pregnancy (24th to* 42nd weeks)

pregnancy regression line is significantly different from that of the normal pregnancy line

$$F=\frac{(s_{b_1})^2}{(s_{b_2})^2}=1.72, \quad P>0.01$$

If regression analysis of prolonged pregnancy is performed for primiparous only then log Y_2 = 2.3328–0.0042 X_2 (N = 105) The intercept is thus somewhat smaller in primiparous, and the fall somewhat larger Regression analysis of determinations from the 294th day onwards (log Y_2 = 3.0341–0.0031 X_2, N = 68) gives a line virtually the same as that from analysis of all values from the 40th to the 46th weeks. This indicates that the decrease was already established in the 40th to 42nd weeks.

In all, eight plasma oestriol determinations were below the normal limit for the 42nd week of pregnancy (< 58 µg/litre Fig. 1) Urinary oestriol excretion was also low in these patients. Seven of the values stem from repeated studies in two patients, who both gave birth to babies showing no evidence of asphyxia. The eighth and lowest value was found in the mother of the only baby which died—following operation for co-arctation of the aorta. All eight samples for analysis were taken before the onset of labour

In the four cases of neonatal asphyxia, no value of plasma oestriol was below 58 µg/litre. The last blood samples were taken 2, 3 6 and 9 days before delivery In the two mothers with the most severely affected babies (Saling 6, and Apgar 5) falls in oestriol concentration had been recorded but the other two mothers had constant levels. Urinary oestriol excretion fell in one mother (baby—Apgar 5) but again no value was below the normal range.

Fig. 2 shows the individual urinary oestriol values plotted against the day of pregnancy on which collection of the urine specimen was completed. Low values corresponded with those mentioned for plasma oestriol.

Fig. 3 shows a significant correlation between plasma oestriol and urinary oestriol in prolonged pregnancy together with the 95% confidence intervals. The stippled lines give the correlation in normal pregnancy There is significant difference between the two regression lines.

DISCUSSION

The demonstration of a significant fall in plasma oestriol concentration during the concluding weeks

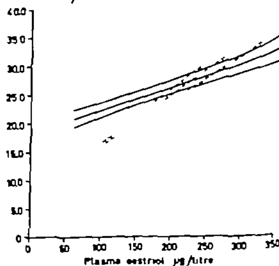


Fig 3 The regression line and 95% confidence intervals for correlation between oestriol concentration in plasma and excretion *in urine during prolonged pregnancy* (Y = 18.5716 + 0.0401 X N = 146). The dashed lines give the corresponding correlation in normal pregnancy (Y = 8.3443 + 0.0853 X N = 59).

of prolonged pregnancy cannot be taken to indicate that prolonged pregnancy *per se* entails risk for the fetus. Oestriol concentration, in this instance, is not necessarily a parameter of placental oxygenation of the fetus, or of placental metabolic functions. It is not known, in the first place, why oestrogen production and metabolism increases so markedly during pregnancy

Fig. 3 shows significant correlation between plasma oestriol and urinary oestriol in both prolonged and normal pregnancy but there is significant difference between the two regression lines. With the reservation that the proportions of the various oestriol conjugates in plasma and urine are different, the regression lines can be taken as an expression of the renal clearance of oestriol (4, 5 19). If the sections of the curves, where the confidence intervals do not overlap (plasma concentration < 180 μg/litre) are considered, it can be read from the regression lines that, at the same plasma concentration there will be greater urinary excretion of oestriol in prolonged pregnancy than in normal pregnancy i.e. that renal oestriol clearance increases in prolonged pregnancy

An opposite conclusion could be drawn from that section of the curves representing a plasma concentration > 310 μg/litre but the small number of observations (six) invalidates such a conclusion

Increased renal clearance of oestriol might be expected following increased production (relative or absolute) of oestriol-16-glucosiduronate or oestriol-3-glucosiduronate because these two conjugates are much more rapidly excreted by the kidneys, than the other two conjugates also present in the blood oestriol-3-sulphate and oestriol-3-sulphate 16-glucosiduronat (15 31) Thus falling plasma oestriol concentrations might possibly reflect only an increased proportion in the blood of those oestriol conjugates most rapidly excreted—the glucosiduronates

Urinary excretion, however did not increase after the 40th week Rather was there a tendency towards reduced excretion (Table IV) It seems probable therefore that oestriol production reduces in prolonged pregnancy I itself a shift towards production of greater quantities of oestriol glucosiduronate could reflect reducing oestrogen metabolism because neither oestriol-16-glucosiduronate nor oestriol-3-glucosiduronate is convertible either to free oestriol or to oestriol-3-sulphate (16). Furthermore oestriol-3-sulphate formation reflects greater metabolic activity than does formation of the glucosiduronates (7).

The experience that falling oestriol concentrations (both in plasma and urine) in pregnancies up to term are associated with an increased fetal risk, and the demonstration that low urine oestriol and fetal acidosis are correlated (11), both indicate that even apparently uncomplicated cases of prolonged pregnancy should be regarded as conditions in which impairment of function of the feto-placental unit is likely

Plasma oestriol studies possibly allow a better physiological evaluation of the condition of a patient with prolonged pregnancy than do urinary oestriol studies. A low plasma concentration is very frequently associated with fetal asphyxia (10)

CONCLUSIONS

Whereas plasma oestriol in normal pregnant women increases in concentration towards term, when pregnancy is prolonged, but otherwise uncomplicated, the plasma oestriol concentration falls after term. Urinary oestriol excretion also falls despite an apparently increased renal clearance of oestriol. The falling oestriol concentrations probably indicate, therefore, that even in uncomplicated prolonged pregnancy the feto-placental unit is in a continuous functional decline.

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LAPAROSCOPY IN SUSPECTED ECTOPIC PREGNANCY

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Abstract. In order to assess the value of laparoscopy as a routine procedure in suspected ectopic pregnancy (E.P.) a study was made on 489 laparoscopies performed on this indication. The laparoscopy was technically successful in 476 cases (97.3%). Only one of 166 E.P. was overlooked. A false diagnosis of E.P. was made in 6 of 312 cases without ectopic gestation. Thus, the total diagnostic accuracy in successful laparoscopies was 98.5%. Combining swiftness and exactitude and covering the whole field of different conditions where suspicion of E.P. arises, laparoscopy is superior to other diagnostic procedures. It is concluded that routine use of laparoscopy in suspected E.P. is justified. Apart from fulminant cases in which it is contraindicated, laparoscopy should be regarded as the method of choice to diagnose as well as to exclude E.P.

In suspected ectopic pregnancy (E.P.) laparoscopy is an excellent diagnostic procedure. This was stressed more than thirty years ago by Hope (7) and others (2, 15). Several comparatively large series of cases (5, 14, 20) and handbooks (1, 11, 18, 19, 21) confirm the statement.

However, it is important to know the accuracy of laparoscopy not only in expert hands but also as a routine procedure.

In our department laparoscopy has been in use since 1948 with increasing frequency. Soon, junior members of the staff also were permitted to perform laparoscopic examinations single-handed, when sufficiently trained to master the technique.

MATERIAL

In order to assess the diagnostic value of laparoscopy as a routine-procedure in suspected E.P. we investigated the laparoscopies made on this indication during 1950–1969. Out of a total of 3284 laparoscopic examinations, 489 were indicated by suspicion of E.P. (14.9%).

Corpus luteum haemorrhage, ruptures (C.L.R.) and large haematomas (C.L.H.), are similar to E.P. from the diagnostic point of view. They do not differ regarding symptoms and signs and are also similar as to the intra-abdominal condition (8). Therefore we have classified them with E.P. in assessing the diagnostic scope of laparoscopy. Hence such cases are not counted as diagnostic errors when regarded as E.P. by the laparoscopist.

According to *the diagnoses made at laparoscopy* the cases are divided in groups (Table I) which are first commented upon separately.

Group 1. Intraabdominal haemorrhage

Blood entirely blocking the view of the adnexae was found in 62 cases (12.7%). When confronted with gross intra-abdominal haemorrhage, the laparoscopist either did not trouble to localize the source of bleeding or could not find it. In such cases laparotomy was always performed immediately. 54 E.P. and 7 C.L.R. were found. In one patient, besides the blood, only an intrauterine pregnancy was revealed. Probably the blood had emerged from the trocar-puncture of the abdominal wall. The after-course in this case was normal. The patient went to term and had a normal delivery with a living child.

Group 2. Ectopic pregnancy visualized

In 120 cases (24.5%) the laparoscopist was able to locate what he considered an E.P. and at once proceeded to laparotomy. The operation revealed 111 E.P., 4 C.L.R., 4 cases of other pathology and in one case nothing abnormal except a small pool of blood in the pouch of Douglas.

There was no blood in the abdominal cavity in 8 cases of E.P. (7%). Obviously E.P. of this kind cannot be diagnosed by vaginal puncturing of the pouch of Douglas.

Among the 4 patients with other pathology which at laparoscopy were falsely diagnosed as E.P. the mistakes regarding one case of ovarian cyst with torsion of the pedicle and one of haematosalpinx are unimportant from a practical point of view as operation is indicated anyway. At first sight it is often impossible to distinguish between a discoloured adnexal torsion and a tubal pregnancy. Only when the twisted pedicle proper is searched for and seen, can a firm diagnosis be obtained. Also a haematosalpinx may closely resemble a tubal pregnancy. On the other hand, 2 cases of salpingitis and one case, where nothing except blood was found, are obvious mistakes. In the last-mentioned case the source of bleeding may have been

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DISCUSSION

When deciding the reliability of laparoscopy as a diagnostic tool in suspected E.P one has to consider (*a*) how often E.P is overlooked or is not possible to diagnose when present, (*b*) the frequency of a false diagnosis of E.P when absent, and (*c*) both together or the total diagnostic error.

The percentage of diagnostic failures depends upon whether only the technically successful and diagnostically suitable laparoscopies are considered or whether also those cases in which a laparoscopy was attempted but a laparoscopic diagnosis could not be obtained are included (group 8).

The figures will of course be different when calculation is made for E.P only for the number of E.P and corpus luteum haemorrhage taken together or if the cases of erroneous diagnosis are related also to the other cases.

(*a*) *E.P overlooked or not possible to diagnose*

In technically successful and interpretable laparoscopies an E.P was overlooked only once in 166 cases of E.P (0.6%). If the 3 cases of E.P in group 8 are included, however, the failure rate becomes 4 in 169 cases (2.4%). Of 478 successful laparoscopies, i.e. excluding group 8, one E.P or only 0.2% was overlooked. In the whole series of 489 cases 4 E.P or 0.8% were missed.

As stated earlier in the present paper, corpus luteum haemorrhage is so similar to E.P from a diagnostic point of view that it should not be considered as a diagnostic error. Consequently it should also be taken into account when not diagnosed.

When the cases of corpus luteum haemorrhage are added to those of E.P only the beforementioned case of E.P and none of corpus luteum haemorrhage was overlooked. In 199 cases, excluding group 8, this makes 0 %. Including group 8 the correct diagnosis was missed in 4 E.P and 3 cases of corpus luteum bleeding in 205 cases, or as much as 3.4%. In all successful laparoscopies the figure is the same as for E.P only. In the whole series 7 cases in 489 or 1.4% were not diagnosed.

(*b*) *False diagnosis of E.P*

A false positive diagnosis of corpus luteum haemorrhage was never made. A false diagnosis of E.P was made in 6 cases. In 312 non-ectopics excluding group 8 and 320 including group 8 the percentage of falsely diagnosed E.P 1.9%. When the cases of corpus luteum haemorrhage are added to the cases of E.P the percentage of false E.P 2.2 in 279 excluding, and 2.1 in 284 cases including group 8.

(*c*) *Total diagnostic error*

Excluding group 8, when laparoscopy failed as diagnostic tool, a wrong diagnosis of E.P was made 7 times in 478 cases (1. %). In group 8 there are 3 E.P

Table II. *Delay in diagnosis*

	1950–54	1955–59	1960–64	1965–69
Laparoscopies on suspicion of E.P	20	50	180	239
Total number of E.P	72	100	114	125
E.P -laparotomy without laparoscopy	63	73	42	39
Delayed diagnosis	23	14	8	6
Per cent delayed of total E.P	32	14	7	5

who were not diagnosed by laparoscopy. If these are included, laparoscopic diagnosis failed in 10 of 489 cases (2.0%).

Taking also 3 cases of corpus luteum haemorrhage in group 8 into account, there are in addition to the 7 diagnostic errors 6 cases not diagnosed by laparoscopy which gives a total diagnostic *failure of 13 cases in 489* or 2.7%.

During the 20-year period 411 cases of E.P were treated in our department. Without preceding laparoscopy 219 patients *having* E.P had a laparotomy sooner or later after admission. In 13 of these the diagnosis was not previously suspected.

The number of such directly laparotomized cases gradually *diminished* as more and more laparoscopy was recognized as the method of choice in doubtful cases.

While by laparoscopy an immediate decision can be made whether or not a case of suspected E.P should be treated by laparotomy most other diagnostic measures constitute a more or less time-consuming procedure, resulting at times in considerable delay before a decision is made. In those of our cases which underwent operation without preceding laparoscopy we have considered one week and more between admission and laparotomy as a delayed diagnosis. As much as 6 weeks delay occurred in isolated instances and two weeks was not uncommon. As seen from Table II the number of delayed diagnoses has gradually diminished along with the increase in frequency of the laparoscopic examinations. Obviously liberal use of laparoscopy reduces the instances of delay to very small number.

Laparoscopy in suspected E.P has certain limits. It is *contraindicated* in fulminating cases, where it is unnecessary and even dangerous. Thus laparoscopy is of course out of the question in

Table I *Diagnosis at laparoscopy*

		%
1 Intraabdominal haemorrhage	62	12.7
2. E.P visualized	120	24.5
3 C.L.R. and C.L.H.	22	4.5
4 Retrograde menstruation	11	2.2
5. Other pathology	53	10.8
6. Intrauterine pregnancy	92	18.8
7 Normal abdominal cavity	118	24.1
8. No diagnosis	11	2.2
Total	489	

the trocar-puncture or possibly retrograde menstruation.

Group 3 C rpus luteum haemorrhage

In 22 cases (4.5%) 18 C.L.R. and 4 C.L.H. were diagnosed. Only 8 of the 18 ruptures underwent laparotomy If the bleeding has stopped at most aspiration of the blood is indicated. This conservative attitude was justified by the aftercourse in every case. Of the 4 patients who had haematomas, one had laparotomy performed. The others were treated conservatively without complications.

Group 4 Retrograde menstruation

In 11 cases (2.2%) the laparoscopist had seen blood oozing from the fimbriated ends of normal tubes, and perhaps a small or relatively large pool of blood in the pouch of Douglas, everything otherwise being normal. None of these patients was operated on. In such cases puncture of the pouch of Douglas from the vagina evidently can cause diagnostic mistakes and unnecessary operations.

Group 5 Other pathology

Pathology other than E.P and corpus luteum haemorrhage was diagnosed in 53 cases (10.8%). In no less than 35 patients further operation was not found to be indicated, and they were spared exploratory laparotomies. There were 31 cases f salpingitis, one of ppendicitis, 11 of endometriosis, one of uterine fibroid, 8 of different kinds of ovarian cysts, and one case of haematoma in the broad ligament without connexion with the tube or ovary The last mentioned patient, the one with appendicitis, 6 patients with endometriosis, 3 with salpingitis and 7 with ovarian cysts were perated on immediately The patient with the uterine fibroid had laparotomy si months later when she reappeared with necrosis of the tumour

Group 6 Intrauterine pregnancy

When intraabdominal pathology could be excluded, intrauterine gestation was diagnosed by the laparoscopist in 92 patients (18.8). In 23 of these abortion occurred of which 16 were before and 7 after the laparoscopy as could be concluded by examining the case sheets. One of these women aborted blighted ovum and 4 had missed abortions. In addition 5 women had legal abortions some time after laparoscopy We quite agree wi Hope (7) who writes: "The danger of aborting is le likely than following laparotomy In fact, the risk o laparoscopy inducing abortion seems to us insignifica or almost entirely absent.

A large number of disturbed intrauterine pregnanc is of course to be expected as the different symptoms an signs can be very similar to those of E.P (17) and ther fore indicate laparoscopic examination.

The majority (58) of the patients in whom an intr uterine gestation was found were delivered of live childr after uneventful pregnancies. With the exception of tw premature deliveries all went to term. One prematu child with a birthweight of 1 500 g was born five mont after the laparoscopy and died. The other prematu child survived. It was born 7 / months after the lapar scopic examination.

In the remaining 6 patients the aftercourse is unknow and they are presumed to have delivered elsewhere o aborted.

Group 7 Normal abdominal cavity

In 118 cases (24.1%) nothing abnormal and no signs o intrauterine gestation were seen in the abdominal cavi However of these patients 29 were in fact pregnant, were delivered at term with living children, 19 evident had aborted before laparoscopy and 3 subsequently Among the latter one patient had missed abortion an one aborted a blighted ovum.

To group 7 belongs the only case where the laparo scopist missed an E.P Three weeks after the laparoscop the patient was operated on for intestinal obstruction A ruptured tubal pregnancy was found.

The remaining 89 patients with no pregnancy and nor mal abdominal cavity had no complications.

Group 8 No diagnosis

I 11 cases (2.2%) the pelvic organs could not b inspected and no diagnosis regarding presence or absenc of E.P could be obtained The laparoscopy as hamper by technical defects of the equipment in 3 cases, pre peritoneal insufflation of CO in 4 cases, and by ad hesions or omentum blocking the iew or obesity in cases. Mishaps concerning the endoscopic instrument are at times inevitable in the wear and tear of daily use Widespread adhesions and obesity can be unsurmountabl obstacles, but prepentoneal insufflation causing emphysem should be avoided by carefully making sure that the insuf flation-cannula has penetrated to free abdominal cavity before insufflation is begun.

Laparotomy was performed in 8 of the 11 patients The laparotomies revealed 3 cases of E.P one f C.L.R., of C.L.H one of salpingitis and one of intrauterine pregnancy The remaining 3 patients who ere kept under observation had no complications.

The only somewhat serious complication in the entire series occurred in one of the E.P mentioned Because of adhesions bet een the intestines and the abdominal wall the trocar of the laparoscope-sheath penetrated into the gut. After operation, including suture of the intestine, the patient had a smooth recovery

including additional diagnostic measures. Laparoscopy allows a firm diagnosis in such cases with as well as without blood in the abdominal cavity when puncturing the pouch of Douglas gives no reliable information. Laparoscopy can be used when culdoscopy and culdotomy are unsuitable.

Exploratory laparotomy can be avoided. Unnecessary laparotomies are almost eliminated, when using laparoscopy

By laparoscopy a diagnosis is obtained at once As the patient is already in proper position and prepared for laparotomy operation, when indicated, can be performed immediately

Erroneous diagnoses are infrequent. In cases of E.P its presence is demonstrated in almost 98 %. In a few cases of suspected E.P the laparoscopist will make a false diagnosis of E.P., which, when not present, is excluded also with nearly 98 % exactitude The combined diagnostic error in confirming and excluding a diagnosis of E.P is 2.7 %

The promptness of laparoscopic diagnosis, making expectancy and various other more or less elaborate diagnostic measures superfluous, saves patients from worry and discomfort and saves time and money

In a great number of patients with suspicion of E.P laparoscopy reveales no intraabdominal pathology I our series this occurred in over 40 Generally such patients can be sent home safely ery soon, in the majority of cases after one or two day

It is concluded that a generous use of laparoscopy as a routine in suspected E.P is justified Apart from fulminant cases, in which laparoscopy is clearly contraindicated and unnecessary it should be regarded as the method of choice to diagnose as well as to exclude E P

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In cases less acute and severe in which an urgent operation is not immediately called for but having symptoms and signs strongly suggesting E.P laparoscopy may seem superfluous. However in cases of this kind inviting laparotomy without additional diagnostic measures, the diagnosis of E.P is at times wrong. This is even more so in more doubtful cases when a suspicion of E.P however less pronounced can not be rejected. In our series there were 34 cases of other pathology not indicating laparotomy and in no less than 210 cases (43%) of the whole series *there was nothing pathologic seen in the abdominal cavity*

A great advantage of laparoscopy is the possibility to be able to exclude E.P at once. When the laparoscopic examination does not indicate laparotomy the patients can be treated conservatively or be sent home safely without having to wait for other measures aiming at proving or disproving a diagnosis of E.P Exploratory laparotomies will be superfluous.

Founding the diagnosis mainly on the history the symptoms and the signs result in a comparatively low degree of accuracy in suspected E.P The figures of Marchetti Kuder & Kuder (10) exemplify this. Out of 219 cases of suspected E.P including those where a diagnosis of E.P was entertained, however remotely a correct diagnosis was made in only 56 6%

Such subsidiary measures in making a diagnosis of E.P as pregnancy tests, hysterosalpingography arteriography and curettage for histologic interpretation have been dealt with at length in the literature As is wellknown all these methods have a more or less limited value They generally imply a loss of time in comparison with laparoscopy

So far we have not discussed the other short cuts to the diagnosis of E.P culdoscopy (5 13) colpotomy (3) and puncturing the pouch of Dougals.

Originally in 1948 we started by using culdoscopy (17) in a few cases with good results. For many reasons, however (16) we soon preferred laparoscopy It gives a better survey of the abdominal cavity and can be used when there are masses blocking the pouch of Douglas and contraindicating culdoscopy

Colpotomy definitely involves some risk of infection and gives a rather narrow field of inspection. We do not think that it can compete with the endoscopic methods even though series with good results (3) have been published.

Puncturing of the pouch of Douglas has a high degree of accuracy (4 9) but apart from showing the presence or absence of blood in the pelvic cavity it gives no information. We admit that it could have given the indication for laparotomy in our group 1 and most cases in our group 2. In retrograde menstruation (group 4) puncture would have been misleading causing unnecessary laparotomies. In unruptured E.P culdocentesis gives a negative result.

Regarding unnecessary laparotomies on suspicion of E.P the large and comparatively recent series of Hall & Todd (6) is representative. In 500 cases of suspected E.P there occurred 46 in which the preoperative diagnosis of E.P proved at laparotomy to have been in error This is no less than 22% of their proven cases of E.P Of the 46 laparotomies 33 or 6.6% of the whole series were considered completely unnecessary In our series laparotomy was unnecessarily made in 3 cases of salpingitis, 2 with an intrauterine pregnancy and one case with a normal abdominal cavity These 6 cases constitute only 1 2% of the whole series.

According to the results of the present series the shortcomings and merits of laparoscopy as a routine in suspected E.P can be summed up as follows.

In a small percentage (about 2%) no diagnosis regarding the presence or absence of E.P can be made.

Comparatively serious complications are quite exceptional. There was only one (an intestinal lesion) Fatal complications can be disregarded.

Combining swiftness and exactitude and covering the whole field of different conditions where suspicion of E.P arises, laparoscopy is superior to other diagnostic procedures In these respects taken together laparoscopy by far surpasses the founding of a diagnosis on symptoms and signs,

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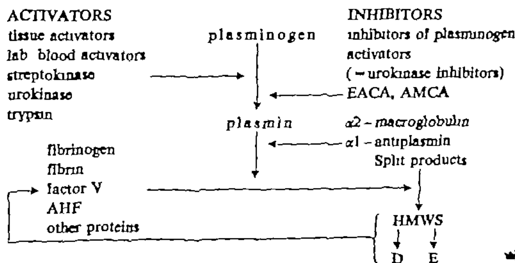
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Gynaecologists

Menorrhagia may be caused by an increase in local fibrinolytic activity Cyklokapron reduces menorrhagic haemorrhages by an average of 50%

Women with average menstrual blood losses of over 80 ml have higher concentrations of plasminogen activators in the endometrium than those with lower blood losses. The resultant increase in local fibrinolytic activity is inhibited by Cyklokapron *The recommended dosage of* Cyklokapron in menorrhagia is *1* g 3–6 times daily for 3–6 days With a dosage of 3 g daily Nilsson and Rybo noted reductions in bleeding *of 38 % compared with control cycles. With* twice this dosage bleeding was reduced by *51 %. None of the 36 patients participating in the trial* were obliged to discontinue treatment as a result of side-effects.

Reference *NILSSON L., RYBO G* *Treatment* of menorrhagia with an antifibrinolytic agent, tranexamic acid (AMCA) A double blind investigation Am J Obstet. Gynecol 110 (1971) *p 113*



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THE ENGAGEMENT OF THE FOETAL HEAD IN THE UTERUS WHEN THE VERTEX PRESENTS

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Abstract In earlier investigations the author demonstrated that the head to cervix pressure both during and between contractions in labour is greatest at the equator of the foetal head and that the head to cervix pressure decreases towards the lower pole of the foetal head. These pressure conditions explain the biomechanics of cervical dilatation during labour under different conditions. The author has used small flat strain gauge receptors sited between the foetal head and the uterine wall. In the present investigation the head to cervix pressure is also measured above the equator of the foetal head. Between the contractions the head to cervix pressure decreases progressively above the equator of the head and finally the amniotic fluid pressure is measured. In some cases, especially primigravidae, the head to cervix pressure curves rise very steeply at the beginning of contractions but as the amniotic pressure approaches its maximum, the head to cervix pressure decreases and ultimately falls to the same level as the amniotic fluid pressure. In other women, especially multigravidae, the increase of the head to cervix pressure at the beginning of the contraction is less, but usually the pressure at the beginning of the contraction increases more rapidly than the head to cervix pressure below the equator. During the contractions the amniotic pressure is measured first at high level and at higher amniotic pressures successively towards the equator. The findings are supported by model experiments using a wooden sphere over which a rubber sheath has been drawn. The investigations explain the engagement of the foetal head in the lower part of the uterus. The importance of the engagement of the foetal head when the vertex presents is related to the influence upon the friction between the foetal head and the uterine wall and the mechanism of cervical dilatation. The engaged foetal head prevents the outflow of amniotic fluid after rupture of the membranes and minimizes the risk of prolapse of the umbilical cord.

Engagement of the foetal head is commonly defined as the descent of the widest diameter of the head below the brim of the true pelvis. The clinical importance of this is well known and failure of descent may occur in association with contracted pelvis, placenta praevia, cervical fibromyoma or an ovarian tumour. Before engagement the foetal head is usually mobile in relation to the pelvis. By analogy the relationship of the head to the uterine wall may be similarly defined as "engaged in the uterus" when the lower part of the uterine wall is closely applied to the foetal head. In earlier papers the author has demonstrated that the head to cervix pressure both during and between the contractions is highest at the largest circumference of the foetal head and that the head to cervix pressure decreases towards the lower pole of the foetal head. The pressure at its highest may be 3–4 times the amniotic fluid pressure and the pressure gradients at levels above the equator of the foetal head may be important in explaining the engagement of the head in the uterus. The aim of the present investigation is to study these pressure conditions. The cervical canal is similar to a cylinder and may be compared with a rubber tube with slight funnelling from above downwards. The clinical observations reported here are supported by model experiments using a wooden sphere in a rubber tube and carried out in collaboration with Holmlund.

METHOD

The method for intrauterine tokometry developed in collaboration with Ingelman-Sundberg and Lindgren (1953 and 1955) was used. The sensitive part of the tokograph (Fig. 1) consists of a strain gauge, enclosed in a circular chamber measuring 1.8 cm in diameter. The gauge is fixed to the central part of a membrane placed inside the chamber and attached to the inside wall. A small piston, 0.5 cm in diameter rests on the strain gauge. The space provided for the piston is 0.6 cm

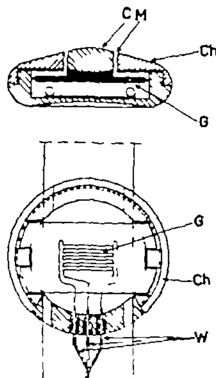


Fig 1 Schematic drawing of the intra terine tokograph. *C* Cylinder *Ch* chamber *M* distance between cylinder and chamber *G* strain gauge *W* wires.

in diameter so as t lea a margin of 0.05 cm around it. The superior surface of the piston is level with the uppe surface of the chamber with a curvature radius of 5 cm and that of the inferior conca surface of 4 cm, th former radius corresponding to the mean radius f a foetal head f normal size The distance from th conve to the concave surfaces is 0.4 cm. Eithe one four tokograph are attached to a 1 cm broad band of *flexible steel with the inferior conca e surface adjacent* to the band. Thus, the apparatu can be introduced easily int the uterine cavity nd yet does not act as a lever When a single tokograph is used it is placed at the end of the steelband. When fou tokographs are used they are placed one t the end, one t 15 cm, one at 18 cm and one at 1 cm distance from the end. The whole instrument is enclosed in a th rubbe sheath. The form of the foetal head below the largest circumference (the equator) corresponds to an irregula ellipsoid Thus foetal head of erage size with a biparietal diameter of 10 cm at the equator has horizontal radius of 5 cm nd longitudinal radius of bout 7 cm (Fig 2) The dist nce from the low r pole of such head to the equato is 9.6 cm The length of the cervical tissue which is dra upwards during the progress of the first stage ncreases from 0.5 cm betwee 1 and cm to 4.3 cm between 9 and 10 cm cervical dilatation. Thus, from 7 cm of cervical *dilatation until full* dilatation 6.3 cm of cervical tissue shall be dra n pw rds

The apparatus is introduced thro gh the cervix and placed between the foetal membranes and the uterine wall. The receptor can either be turned toward the uterine wall or tow rds the foetal membranes

The position of the different receptors as estimated in different manners. In some cases the lowest receptor was placed and measured the head to cervix pressure at the external os and was repositioned as cervical dilatation progressed. At or abo e the equator of a head of normal size the pressure was measured first at about 7 cm cervical dilatation. In other cases the receptor as located in relation to the equator I e the region of highest head *to cervix pressure Since the distance bet een the receptors* is constant (3 cm) the levels of all receptors can be *defined* in relation to the reference point. The apparatus is also graduated with a / cm scale. The error of the estimated position f th receptor has been calculated to ±0.5 cm. Because of the curvature of th normal foetal head the radius above the equator decreases from 5 cm to about cm. With regard to the thickness of the receptor (0.4 cm) the pper point of contact between the head and cervix as measured by the tokograph can theoretically be 1.0 cm higher than the actual contact poi t. The weakness of the uterin tissue and the increase of the width of the cervical canal upwards reduces this error

MATERIAL

The series comprises 18 women at term. All ere at normal labour and deliv red with anterior ertex presentation and without any clinical evidence of contracted pelvis. Eleven were nulliparae. The birth weights aried between 770 g and 4 000 g. I four secundigravidae measurements were made before the onset of labour and in

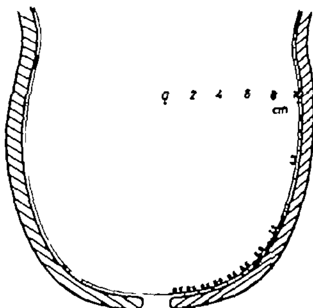


Fig Schematic representation of the low part of the uterus t the onset of labour The figures at the equator f the foetal head represe t cervical dilatation estimated in the usual way The figures along the terine ll represent the length of dilating cervical tissue in centimetres The amount of cervical tissue to be dilated for each centimetre of dilat tion measured horizontal to the foetal head increases as labour progresses.



Fig. 3. Intrauterine pressures recorded before the engagement of the foetal head in the lower part of the uterus. *A*, From tokograph above the foetal head, *B* tokograph 2 cm below the equator of the foetal head, *C*, tokograph 5 cm below the equator of the foetal head. All curves show the same pressure but disturbances of the motility pattern are more evident in *B* than in *C*.

all the foetal head was movable in the uterus. In one case the membranes ruptured during the recording. In the other women the recordings were made from 3 cm of cervical dilatation until full dilatation. In six cases the membranes were already ruptured. In nine women the receptor was turned towards the uterine wall and in five towards the foetal head. In addition to these normal labour recordings, primigravida with spasm of the lower uterine segment was also studied.

RESULTS

Head to Cervix Pressure below the Equator

Before the onset of labour

In all four women the foetal head could be ballotted on abdominal palpation. The same pressures were recorded below and above the equator of the foetal head. In three women the pressure increases were low during the pre-labour contractions. In one women more marked increases in amniotic pressure was observed during contractions (Fig. 3). After engagement the pressure increased at the equator of the foetal head.

During labour

In earlier investigations the head to cervix pressures were measured under different conditions. In more than 300 cases studied, usually the head to cervix pressure was greatest at the equator of the foetal head and decreased towards the lower pole of the foetal head. In the present studies the pressures below the equator were measured in all cases and the following is a typical example.

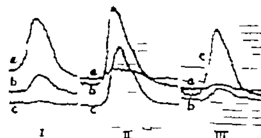


Fig. Pressure curves in normal labour. The head to cervix tokographs with the receptor facing the uterine wall. Membranes ruptured. Secundigravida. Recordings by one tokograph placed 6 cm above the external os (*a*), one 3 cm above the external os (*b*), and one just inside the external os (*c*) in Fig. 4. I. 1 cervix dilated to 7 cm; II. cervix dilated to 9 cm; III. cervix dilated to 10 cm. For further explanation see text.

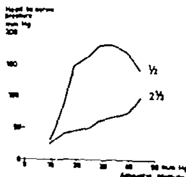


Fig. 5. Diagram of the head to cervix pressure during contraction in primigravida. The head to cervix pressures measured with the receptor turned towards the uterine wall. + / / cm above the equator of the foetal head. −2 / 2 / cm below the equator. Note the rapid increase of head to cervix pressure at + at the beginning of the contraction and the decrease of pressure at higher amniotic pressure.



Fig. 6. Pressures in the uterine cavity. *Upper curve:* amniotic pressure. Intrauterine tokograph with the receptor turned towards the foetal membranes. *Middle curve:* Intrauterine tokograph with the receptor turned towards the uterine wall. *Lower curve:* amniotic pressure measured according to Williams and Stalworthy. The middle tokograph records higher pressures than the amniotic pressure recorded by intrauterine tokometry and the method of puncture of the amniotic cavity. (From Ingelman-Sundberg & Lindgren, J Obst Gynaec Brit Emp 6, 629, 1955.)

In this patient, a secundigravida, the positions of the receptors were assessed from the lowest tokograph which was placed just within the external os (Fig. 4). The measurements were taken at 7–10 cm of cervical dilatation. The pressure at the equator of the foetal head is recorded by the highest tokograph (*a*) in Fig. 4 I, by the middle tokograph (*b*) in Fig. 4 II and by the lowest tokograph (*c*) in Fig. 4 III. The head to cervix pressure above the equator is recorded by the tokograph (*a*) in Fig. 4 II and by the tokograph (*b*) in Fig. 4 III. The highest tokograph (*a*) in Fig. 4 III records the amniotic pressure.

Head to Cervix Pressure above the Equator

Before rupture of membranes

The head to cervix pressure above the equator was measured in eight women, two being secundigravidae. In six cases the receptor of the tokograph faced the uterine wall. The pressure recordings from one of the patients in seen in Fig. 5. At the level of $+^1/_2$ ($^1/_2$ cm above the equator) one finds first a very rapid increase of pressure up to 30 mmHg amniotic pressure but then the head to cervix pressure decreased. The increase of pressure at the beginning of the contraction is much greater than that below the equator. These findings are in contradiction to the following recordings when the tokograph is turned towards the foetal head and the foetal membranes and after rupture of membranes. Sometimes one can observe a motility pattern superimposed on the pressure curves in the same manner as was observed in an earlier paper (2) when the receptor faced the wall of the corpus before rupture of membranes (Fig. 6).

In two women the receptor faced the foetal membranes and head. In these women, as in the case described above, the resting pressure between the contractions was higher than the corresponding amniotic pressure (amniotic tone) $^1/_2$–1 cm above the equator but during contractions the amniotic pressure was measured (Fig. 7). Two centimetres (the level +2) above the equator the amniotic pressure was recorded throughout the

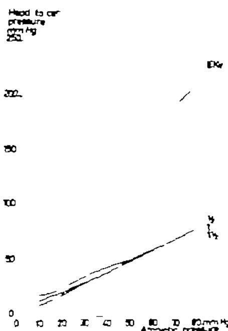


Fig. 7. Head to cervix pressure correlated with the amniotic pressure during the contraction phase of a secundigravida. The head to cervix tokographs turned towards the foetal membranes. Recorded during four contractions at 5–7 cm of cervical dilatation. EI: Equator, +1 and +1 1/2 = 1/2, 1 and 1 1/2 cm above the equator. Note the decrease of amniotic tone upwards and at +1 cm the amniotic pressure is recorded during the whole contraction. At the level +1 the amniotic pressure is recorded from 30 mmHg of amniotic pressure. At the level +1/2 the amniotic pressure is measured from 50 mmHg of amniotic pressure.

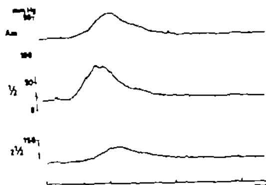


Fig. 8 Normal contraction in primigravida. The head to cervix tokograph with the receptor facing the uterine wall. Recorded at 6 cm of cervical dilatation. *Am*, Amniotic pressure. + / head to cervix pressure / cm above the equator. 2 / head to cervix pressure 2 cm below the equator. Note the steep rise of pressure above the equator. Membranes ruptured.

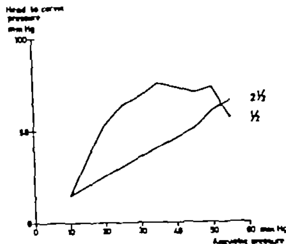


Fig. 9 Head to cervix pressure correlated with the amniotic pressure. Diagram from the contraction phase in Fig. 8. + / / cm above the equator. −2 / 2 / cm below the equator. Note the steep increase of pressure above the equator at the beginning of the contraction and the decrease after the amniotic pressure reaches 35 mmHg.

contraction. The patient whose contractions were recorded in Fig. 7 was a secundigravida. In this diagram the rapid increase of head to cervix pressure at the beginning of the contraction is not seen as in the case described above.

After rupture of membrane

In six women, one of whom was a secundigravida, the head to cervix pressures were measured at different levels. In four women the receptor faced the uterine wall and in three women faced the foetal head. From the equator up to 1 / cm above it in all women the resting pressure was higher than the intraamniotic pressure. With increasing amniotic pressure during contractions the same pressure conditions were obtained as before rupture of membranes when the receptor was turned towards the foetal head. In primigravidae (+ (cm above the equator) the pressure increased very steeply at the beginning of the contraction but decreased at higher amniotic pressure and finally measured the amniotic fluid pressure. This observation, which was the same as was observed before rupture of membranes was confined to primigravidae (Figs. 8, 9 and 10). In secundigravidae, and also in some primigravidae, pressures recordings were obtained similar to those shown in Fig. 7. With increasing amniotic pressure the amniotic pressure was first measured at the highest head to cervix level above the equator and then at lower levels.

Lower uterine spasm

In cases of lower uterine spasm motility pattern was added to the head to cervix pressure curve both below and above the equator of the foetal

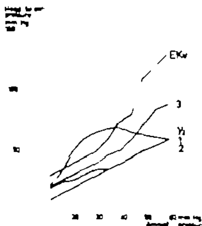


Fig. 10 Head to cervix pressure correlated with the amniotic pressure during the contraction phase in primigravida. The head to cervix tokograph with the receptor facing the foetal head. Recordings during four contractions at 5–7 cm of cervical dilatation. *Ekv* Equator, + +1 and +2: / 1 and 2 cm above the equator. −3: 3 cm below the equator. Note the head to cervix tone at different levels. The highest head to cervix tone is measured at the equator and decreases upwards.

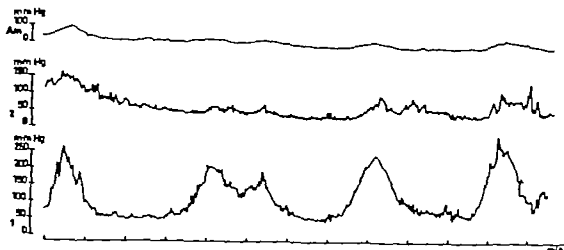


Fig. 11. Lower uterine spasm. The head to cervix tokographs with the receptor facing the uterine wall. *Am*, Amniotic pressure. +... head to cervix pressure cm above the equator. −1, head to cervix pressure 1 cm below the equator. The recording shows spastic contractions below as well as above the equator. For further explanations see text.

head. Spastic contractions were recorded up to 5 cm above the equator of the foetal head. In Fig. 11 recordings from such a case are presented. One can note that the head to cervix pressure below the equator follows the amniotic pressure curves but above the equator this correlation is not so evident.

Model experiments

In order to explain the biomechanics of the engagement of the foetal head in the lower part of the uterus some model studies have been carried out.

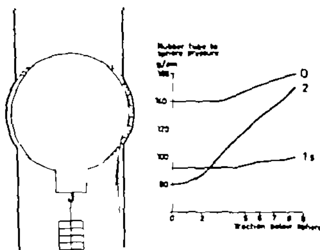
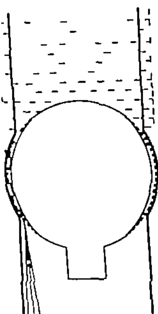


Fig. 12. Diagram of the rubber tube to sphere pressure at different levels of a sphere when increasing traction force was applied to the sphere. 0 = the equator, +2: cm above the equator, −1 ... 1 / cm below the equator. The dotted lines mark the decrease of the diameter of the rubber tube during traction.

The model consists of the rubber tube fixed at its upper end. A wooden sphere was placed inside this tube (Fig. 12). Water could be poured into the tube above the sphere (Fig. 13) and traction could be applied on a hook at the lower pole of the sphere (Fig. 1?). The thickness of the wall of the rubber tube was 0.2 cm and the inside diameter 6.5 cm. The sphere was made of wood, with the diameter of 8 cm. A groove was made in the direction of the meridian of the sphere in order to accomodate the tokograph on the surface of the sphere. The tokograph could be placed at different levels in this groove and its position was related to the largest circumference of this sphere, the equator (0). The distance from the equator was measured outside the rubber tube. Ex. +1 represents a position of the tokograph 1 cm above and −2 a position 2 cm below the equator.

Traction was applied by placing weights on a hook at the lower pole of the sphere (Fig. 12). The sphere did not advance when a traction force of less than 8 kp was applied. With increasing loads the rubber tube above the sphere was stretched and its diameter decreased (marked by *dotted lines in Fig. 12). An increase in the traction* force caused a linear rise in pressure which was greatest above the equator and least below it.

Water was poured into the tube from above and the height of column of water was measured from the top of the sphere by means of a measure outside the tube. The sphere advanced when the column of water above the sphere reached 35–



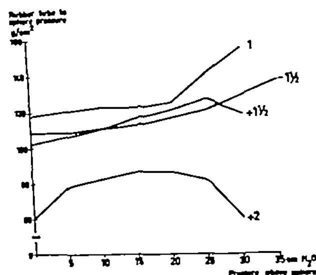


Fig. 13. Diagram of the rubber tube to sphere pressure at different levels of sphere hen increasing pressure of column of water was applied above the sphere. 1 1 cm above the equator −1 1 cm below the equator The curve (+2) is achieved hen the tokograph was applied 2 cm above the equator but with its receptor facing the sphere. Note the dotted lines, corresponding to the distension of the rubber tube above the sphere.

40 cm The driving force of this column of water was 1 7 2 0 kp A rise in the pressure above the sphere caused no linear rise in pressure. Above the equator the sphere-tube pressure rose rapidly when the water pressure reached a head of 5 cm A further rise in the pressure bove the sphere caused a little rise in the sphere-tube pressure. When the pressure rose from 22–25 cm water the sphere-tube pressure gradually fell. Below the equator the pressure was little affected when the head of water was 20 cm But further rise in the water pressure caused a steep rise in the sphere-tube pressure.

The explanation of the obser tion is that with low traction forces, weights or low water pressures abo the sphere the rubber tube is pressed towards the sphere above the equator With higher water pressures the rubber tube is distended and break contact with the sphere above the equator (marked by dotted lines in Fig 13) This results in decreasing sphere to rubber tube pressure bove the equator

CONCLUSIONS

Before engagement of the foetal head in the lower part of the uterus pressures are uniform throughout the uterine ca ity excluding the dif ference of the hydrostatic pressures. After engagement the head to cervix pressure is higher than the amniotic pressure between the contrac tions both below and above the equator of the foetal head During contractions the head to cervix pressure abo the equator increases much more steeply than below the equator in some women. At maximal amniotic pressure the head to cervix pressure above the equator decreases. The explanation is that at the beginning of the contraction the tissue of the uterine wall is stretched and is pressed towards the foetal head above the equator At higher amniotic pressures the tissue of the uterine wall is distended above the equator by the amniotic fluid, which explanation is supported by model studies using a rubber tube which has been drawn over a wooden sphere. The importance of the distension of the uterine wall is the decrease of the friction be tween the foetal head and the uterine wall which mak cervical dilatation easier The longitudinal stretching of the uterine wall as the result of tension as demonstrated by Siener increases the friction between the foetal head and the uterine wall and plains the clinical observations that

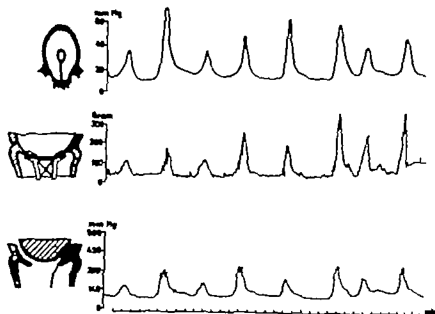


Fig. 14. Simultaneous recordings of amniotic pressure, tangential cervical tension and head to cervix pressure at the largest circumference of the foetal head. *Upper curve*: amniotic pressure (mmHg). *Middle curve*: tension of the cervix (g). *Lower curve*: head to cervix pressure. (From Lindgren & Siener: Am J Obst Gynec 95: 414, 1966.)

vacuum extraction in the presence of a non-dilated cervix is sometimes difficult especially if traction is applied between the contractions (Fig. 14). Such a steep increase of head to cervix pressure above the equator was not observed in all women especially in the case of multiparae. The explanation seems to be the different widening of the uterine wall above the equator. The steep rise in pressure above the equator has only been observed in primigravidae and may be associated with oligohydramnios and dysmaturity. During contractions the amniotic pressure is first measured at the highest point of the head to cervix contact area and after that successively towards the equator of the foetal head.

In normal labour the length of the head to cervix contact area above the equator has been estimated at 1–4 cm without correction for the thickness of the tokograph. The thickness of the tokographs may reduce this height less than 1 cm.

The series is too small to draw any definite conclusions about the differences of the height of the head to cervix area above the equator between the contractions under different conditions. However, no differences were observed if the receptor faced the foetal head before and after rupture of membranes or if the receptor faced the uterine wall after rupture of membranes. The length of the head to cervix area was in these cases estimated at 1.6 ± 0.1 cm. If the receptor was turned towards the uterine wall before rupture of membranes the average length was estimated at 2.6 ± 0.5 cm. The difference may be caused by error of the recording method. Secundigravidae show a length of 3.2 ± 0.6 cm and the primigravidae 1.7 ± 0.2 cm. No differences in the length of the head to cervix area above the equator were found with regard to the degree of cervical dilatation or the size of the foetus. The differences of the head to cervix pressures and the differences of the length of the head to cervix area above the equator may be caused by different amounts of amniotic fluid and a different form of the lower part of the uterus.

In a woman with lower uterine spasm quite different pressure conditions were observed and the length of the head to cervix area above the equator between the contractions was estimated up to 5 cm uncorrected. In addition to its influence on cervical dilatation the importance of the engagement of the foetal head in vertex presentation is that the foetal head prevents loss of amniotic fluid after rupture of membranes and also prevents prolapse of the umbilical cord. The foetal head thus is engaged in the uterus between contractions but not during the contractions in normal labour. In lower uterine spasm the foetal head is engaged in the lower part of the uterus not only between contractions but also during the contraction and explains the delayed cervical dilatation in uterine spasm.

ACKNOWLEDGEMENT

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CHROMOSOME PATTERN IN A PATIENT WITH CERVICAL CARCINOMA IN SITU AND ATYPICAL HYPERPLASIA OF THE ENDOMETRIUM

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Abstract. This report concerns patient with preinvasive cervical carcinoma and atypical hyperplasia in the endometrium. Separate chromosome analyses ere performed on foci of moderate dysplasia and carcinoma in situ in cone biopsy and on material obtained from two curettage specimens. The chromosome picture in the wo cervical areas differed and indicated clonal evolution. The two curettage specimens ere at variance ith the cervical epithelium. The cytogenetic difference between squamous and columnar dysplasia is discussed in relation to the possible pathway of development of malignancy

The female genital tract presents good opportunity for following pathological changes in the epithelial tissue, and the combination of cytology histology and cytogenetic studies is important when considering the development of malignancy In recent years detailed information has been obtained indicating that cytologic examination of the uterine cervix is a reliable and accurate method of diagnosing premalignant changes In the diagnosis of endometrial carcinoma the identification of atypical columnar cells is less reliable A means of improving cytology in the detection of endometrial carcinoma, uterine lavage is under trial in our clinic (to be published) In limited number of patients cytogenetic studies have been performed on material obtained by curettage following uterine lavage. The present report concerns one patient with both squamous and columnar cell atypia in the cytological and histological specimens

MATERIAL AND METHODS

The patient is 43-year-old premenopausal woman without clinical symptoms, except slightly irregular menstrual cycle. She was submitted to the general population screening The vaginal smear contained atypical cells with both squamous and columnar differentiation and gave rise to suspicion of malignancy (Figs. 1 and 2). Prior to cervical biopsy and curettage, the uterine cavity as washed ith isotonic NaCl. The lavage material, collected by nucleopore filter contained groups of slightly atypical columnar cells similar to those observed in the vaginal smear Histologically the endometrium was in the proliferative phase with small areas showing glandular crowding and slight epithelial atypia (Fig 3). Squamous epithelium as not observed in the curettage material. The biopsy specimen from the portio and cervical canal showed carcinoma in situ (CIS) of intermediate type. Because of persistent abnormal cells in the smear cone biopsy and second curettage as performed 10 days later The endometrium was hyperplastic with minor atypia, less than in the previous curettage (Fig. 4). Serial sections of the cone biopsy showed small focus of moderate dysplasia (Fig 5) and in the opposite quadrant circumscript focus of CIS (Fig. 6), without evidence of invasion The patient as in good health 5 month later and she is being subjected to continuing follow-up.

Chromosome analysis was performed on fragments obtained from the two endometrial specimens, referred to as curettage I and curettage II Immediately after the cone biopsy small parts of the epithelium were removed from the squamo-columnar junction and prepared separately from the four quadrants. Metaphases were obtained from tw opposite quadrants and the t different preparations are referred to here as cone-a (dysplasia) and cone-b (CIS) The direct squash technique was used. The method, nomenclature and statistical treatment have been described earlier (4). All the unbroken and well-spread metaphases were counted and hen possible photographed and karyotyped.

RESULTS

The cytogenetic picture in the preinvasive cervical epithelium has been reported earlier (4 Case 11). As is evident from Table I the numerical chro-



Fig   Cone-a. Moderate squamous dysplasia.



Fig. 6. Cone-b Squamous carcinoma in situ. (Figs. 5–6. The specimen for chromosome analysis taken from the area on the right. H & E stain,   750)

analyses were performed in 29 cells, 5 of these were normal diploid and 4 cells, containing 45 chromosomes, had an identical karyotype with loss of one D chromosome (Table II and Fig. 7 *b*). About 50% of the karyotypes had gains or losses of one to 4 C chromosomes, losses of one or 2 D and loss of one G. A few cells had a loss in groups B E17 18 and F In one cell with 46 chromosomes one A3 was missing and in addition one marker was found of the size of a B chromosome, the short arm having only half the length of that in the normal B It is reasonable to suppose that this marker is due to   deleted A 3 The deviations did not exceed 0.5 in relation to the mean karyotype 43 8 in group D the mean chromosome loss was only 0 46 (Table III)

The chromosome pattern in the second curettage (II) differed from that in curettage I. Altogether 50 cells were studied, and those with 46 chromosomes were in a distinct majority (78 %). Also, normal female karyotypes were numerous (Table II Fig 7) 1 the hypodiploid   riant cells losses were found in the same chromosome groups as in curettage I One of the   polyploid cells was analyzed and showed an exact duplication of the normal female karyotype except for one abnormally long, possibly despiralized, chromosome A2 (Fig 7 *d*) In relation to the mean karyotype 45 4 the deviations did not exceed 0 1 in any chromosome type In comparison with the deviations in cone-b the chromosome pattern in the endometrium differs significantly from the CIS population (T ble III)

DISCUSSION

To date many cytogenetic reports are available on human endometrium. It has been stated that the normal endometrium studied in vivo and in vitro has 46 as the modal number Except for a few studies with only 3 –51% of the cells containing 46 chromosomes (3 5 12), most authors found 71–100% cells with 46 chromosomes (7

Table 1 *Chromosome counts in the present material*

Chromosome no.	Chromosome count in specimen			
	Cone-a	Cone-b	Curettage I	Curettage II
39	—	—	1	—
40	1	—	1	—
41	—	—	4	—
42	—	—	4	1
43	—	—	4	4
44	—	—	3	1
45	2	1	7	3
46	5	—	7	39
47	1	1	—	—
48	—	1	—	—
49	—	—	—	—
50	—	3	—	—
51	—	2	—	—
52	—	5	—	—
53	—	1	—	—
54	—	6	—	—
55	—	4	—	—
72	—	1	—	—
79	—	1	—	—
83	—	—	1	—
90	—	—	—	1
92	—	—	—	1
94	—	1	—	—
Total	9	27	32	50









Fig 1 Dyskaryosis of the superficial and parabasal squamous cells

Fig A cluster of atypical endometrial cells. (Figs. 1– Papanicolaou stain, 1 340)

Fig 3 Curettage I. Endometrial hyperplasia with glandular crowding and slight atypia

Fig 4 *Curettage II. Endometrial hyperplasia* with a milder degree of crowding and atypia. (Figs 3–4 H & E stain, 335)

mosome pattern differs in the two preparations. In cone a the 9 cells studied had the maximum cell count at 46, and the 2 cells karyotyped were normal diploids, except for one abnormally long chromosome A1 with decreased spiral density In cone-b the modal population was hyperdiploid (stem-line = 52) In 89 of the cells studied a long subterminal marker was found, which on an average was 45 % *longer than the normal B chromosome* (Fig. 7 *a*)

From the first *curettage (I) 3* cells were studied. As is evident from Table I the maximum cell count was *45* and *46* *and the majority of* the variant cells had counts in the hypodiploid range. *Only* 7 cells (22 %) had 46 chromosomes. One cell contained 88 chromosomes. Karyotype

Table II. *The chromosome characteristics of the modal population*

No deviations in groups A1, A2, and E16

Specimen	2n	Chromosome type A3	B	C	D	E17 18	F	G	Marker	No. of karyotypes
Curettage I	39		−1	−4	−1			−1		1
	40		−1	−4	−1					1
	41		−1	−2	−1			−1		1
	41			−3	−1			−1		1
	41			−2	−1		−1	−1		1
	41			−1	−2		−1	−1		1
	42			−1	−2			−1		2
	43		−1	−1				−1		1
	43			−1		−1		−1		1
	43			+1	−2		−1	−1		1
	44		−1	+2	−1		−1	−1		1
	44				−1			−1		2
	45		−1	+1	−1					1
	45				−1					4
	45					−1				1
	45						−1			1
	46	−1							+1	1
	46			−1				−1		1
	46									5
Curettage II	43			−2	−1					1
	43			−1	−1	−1				1
	43				−1	−1		−1		1
	45			−1						1
	45				−1					1
	45							−1		1
	46									13

frequent in group G. In hyperplasia of the endometrium without and with atypia the normal female karyotype dominated more often, thus indicating more benign condition in comparison to CIS. Reports on analyses of variant cells are still too rare to permit conclusions regarding deviations in particular chromosome groups.

In the present case the chromosome picture in the cervical dysplastic area was similar to that in earlier studies on dysplasia, the modal population was diploid and the two normal karyotypes indicate a stem-line at 46. Unlike this population and contrary to the majority of the published reports of diploid preinvasive lesions, the CIS area had a hyperdiploid mode. As is evident from Table III the deviations differed significantly in the two cone areas. The B-type marker not observed in the dysplastic area, supports the presence of a new

Table III *Mean differences between observed and xpected values*

Specimen	Mean no	Chromosome type A1	A2	A3	B	C	D	E16	E17–18	F	G	No. of karyotypes
Cone-a	44.0											2
Cone-b	51.3	0.7	0.3	0.3	0.6	1.4	0.2		−0.7	−0.6	0.4	10
	72	2.9	0.1	0.1	0.3	3.0	1.4	0.1	−2.3	−0.3	−1.3	1
Curettage I	43.8	0.1	0.1	0.1		0.2	0.5	0.1	0.1		−0	28
	88	0.2	0.2	0.2	1.7	5.4	1.5	0.2	0.4	−1.7	1.7	1
Curettage II	43.4				0.1		0.1		0.1	0.1	0.1	19
	9**											1

Compared th the normal 4 set

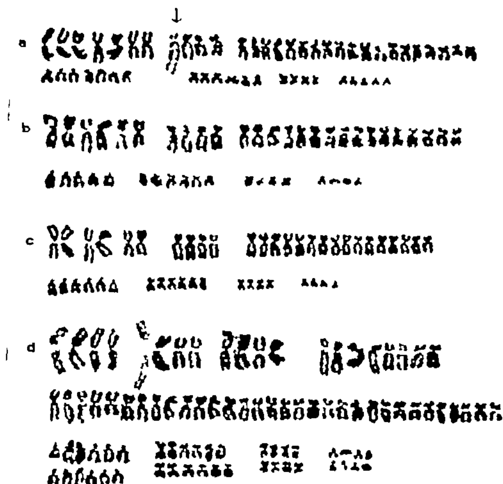


Fig 7 (a) CIS area, karyotype of a stem-line cell, S = 52. Gain of one A1 three C, one D and one G. Arrow B-type marker (b) Curettage I, karyotype of hypomodal variant cell. Loss of one D chromosome (c and d) Curettage II karyotypes f a normal diploid and tetraploid cell, respectively

8 9 10 11) A small proportion of cells was hypodiploid, and hyperdiploid cells were found only accidentally (8 11 14) Between 3 and 10% of the cells were polyploid, almost exclusively in the tetraploid-hypotetraploid range. Because of technical difficulties our knowledge of the chromosome complement in normal cervical epithelium is limited However there is evidence that like the endometrium, the mode is normal diploid (1).

There are obvious differences between the preinvasive cervical lesion and the atypical hyper plastic endometrium. The invasive cervical epi thelium is composed of a mainly diploid and/or tetraploid population, the diploid being somewhat more frequent. Most of the lesions with a diploid modal population have a maximum cell count at 46 The variant cells are usually spread symmetrically in the hypo- and hyperdiploid range (4) The chromosome complement in hyperplasia of the endometrium without and with atypia, studied in vivo and in vitro was almost exclusively diploid (2, 6 9 10 14) There is only one case published relating to a patient with fibroma uteri and slight endometrial irregularity in which the majority of the 21 metaphases had counts in the tetraploid region (9). In the diploid cases a maximum cell count at 46 was predominant but unlike the preinvasive cervical epithelium the variant cells were concentrated in the hypodiploid range, and hyperdiploid and polyploid cells were found only accidentally

In dysplasia and CIS with a diploid mode, submitted to a fairly detailed chromosome analysis, the normal female karyotype was frequent and often represented the stem line of the lesion. In the variant cells gains were observed mainly in group C, and losses in group D and somewhat less

Table II. *The chromosome characteristics of the modal population*

No deviations in groups A1 A2, and E16

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	41			−1	−2		−1	−1		1
	42			−1	−2			−1		2
	43		−1	−1				−1		1
	43			−1		−1		−1		1
	43			+1	−2		−1	−1		1
	44		−1	+2	−1		−1	−1		1
	44				−1			−1		2
	45		−1	+1	−1					1
	45				−1					4
	45					−1				1
	45						−1			1
	44	−1							+1	1
	46			1				−1		1
	46									5
Curettage II	43			−2	−1					1
	43			−1	−1	−1				1
	43				1	−1		−1		1
	45			−1						1
	45				−1					1
	45							−1		1
	46									13

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T ble III *Mean differences between observed and xpected value*

Specimen	Mean no	Chromosome type A1	A2	A3	B	C	D	E16	E17–18	F	G	No. of karyotypes
Cerv-a	44 0											2
Cerv-b	5 3	0 7	0 3	0 3	0 6	1 4	0.2		0.7	0 6	0 4	10
	72	2 9	0 1	0 1	0 1	3 0	1 4	0 1	−2.3	0 3	−1.3	1
Curettage I	4 8	0 1	0 1	0 1		0 2	0 5	0 1	0.1		[illegible]	28
	88	0 2	0 2	0 2	1 7	5 4	1.3	0 2	0 4	1 7	−1 7	1
Curettage II	45 4				0.1		0 1		0.1	0.1	0 1	19
	9*											1

Compared th the normal 4 set

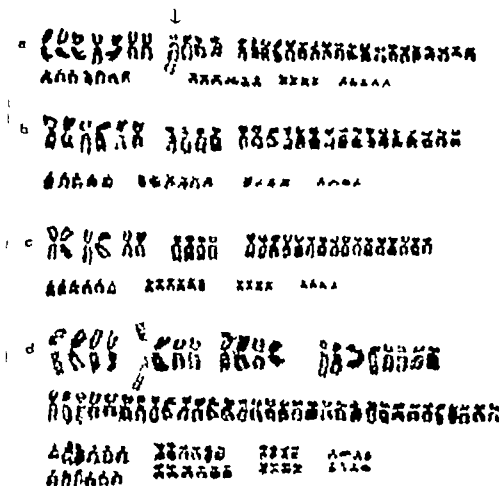


Fig 7 ( ) CIS area, karyotype of stem-line cell, S – 52. Gain of one A1 three C, one D and one G Arrow: B-type marker (b) Curettage I, karyotype f a hypomodal variant cell. Loss of one D chromosome. (c and d) Curettage II, karyotypes of a normal diploid and tetraploid cell, respectively

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clone in the CIS focus. It is thus postulated that the latter area may have originated from a single altered cell.

The chromosome picture in the curettage specimens is more difficult to evaluate, since it is not known from what part of the endometrium the metaphases originated. In both preparations the material was collected from several fragments and thus a comparable situation is presupposed. In curettage II however the cytogenetic data have to be evaluated with care since the specimen was obtained only 10 days after the first curettage. The greater frequency of diploid metaphases is doubtless due to a *normal regenerative process* following the prior diagnostic and therapeutic procedure.

Though non-disjunctional variation would be expected to give a fair number of both hypo- and hyperdiploid variant cells, all cell counts were diploid-hypodiploid The karyotype analysis indicates that the hypodiploid chromosome counts are correct and not caused by an artificial loss during preparation. an identical karyotype was found in four cells with 45 chromosomes (curettage I), possibly representing the start of a hypodiploid side-line. The remaining variant cells showed a trend towards a similar non-random distribution with deviations most frequently in chromosome groups C, D and G

The significance of the cytogenetic difference between squamous and columnar cell atypia is still obscure From pathological experience it is wellknown that dysplastic changes of the endometrium are more often limited to a smaller area and may disappear spontaneously by discharge during menstruation, or are removed altogether by a curettage. Also the preinvasive cervical lesion may represent a more advanced stage in the malignant development. As a matter of fact we still know little about the regress or progress of endometrial atypia, and histological examination alone is not sufficient to predict the development in an individual case This applies especially to the milder forms of endometrial atypia

There is likewise an interesting cytogenetic difference between cervical and endometrial carcinoma. In the former about 60 % have a mode spread in the diploid region, and a second large group has a triploid mode (about 30 %) while only a few cases are tetraploid (4) Analysis of previously published reports on uterine adenocarcinomas shows that the mode in the diploid region is significantly more frequent, only about 10 have a polyploid mode, and these are almost exclusively triploid. Here the chromosome findings permit interesting speculation concerning the development of definite malignancy from preinvasive conditions. In squamous cervical epithelium the progression may be due to several pathways since both diploid and polyploid populations occur frequently in preinvasive lesions. In the endometrial dysplasia the predominance of diploid-hypodiploid cells is manifest. Owing to the *rapid renewal of the endometrial epithelium, polyploid populations* are obviously unable to develop in premenopausal women. This may be one reason for the dominating diploid mode of adenocarcinomas in the uterus. Further studies are required to elucidate this problem.

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SHORT TERM PROGESTOGEN TREATMENT OF ENDOMETRIAL CARCINOMA

Histological, Histochemical and Hormonal Studies

Narve Moe

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Abstract. Thirteen women with adenocarcinoma of the endometrium were treated with standardized doses of hydroxyprogesterone caproate for 3 weeks. Histological and histochemical studies of tumour tissue were performed before and after the treatment. Most of the well differentiated carcinomas showed secretory and acanthomatous changes after the treatment, whereas such changes were more rarely seen in tumours with low degree of differentiation. There was great variability in the enzyme pattern. Alkaline phosphatase activity seemed to decrease during the treatment, whereas acid phosphatase and adenosine triphosphatase activities were mostly unaffected. The serum levels of LH decreased, whereas the plasma level of corticoids and the urinary excretion of oestriol and pregnandiol showed no definite changes during the treatment.

A considerable number of publications have appeared dealing with long-term progestogen treatment of recurrent, advanced or metastatic endometrial carcinoma where the primary tumour or the metastases have shown signs of regression.

However there are only few reports concerning the histological changes after short-term progestogen therapy of previously untreated endometrial carcinoma (2, 7, 8, 11, 20, 21, 22).

The histochemical pattern of primary adenocarcinoma of the endometrium before and after progestogen therapy has been described by Thiery & Willighagen (20) and Vokaer et al (22). In these investigations the doses of progestogens and the duration of treatment varied considerably.

In the present paper histological, histochemical and hormonal studies of primary endometrial carcinoma before and after intensive progestogen therapy with standardized dosage of 3 weeks duration will be presented.

MATERIAL AND METHODS

Thirteen patients with primary adenocarcinoma of the uterine corpus were treated for 21 days with 17alpha-hydroxy-progesterone-caproate (Primolut Depot® Schering), 1000 mg daily or 17alpha-hydroxy-19-nor-progesterone-caproate (Depostat® Schering), 200 mg daily. These doses can be considered as equivalent. Data concerning the patients are listed in Table I.

The diagnosis was based on histological examination of endometrium obtained by curettage. After the hormone treatment, radical hysterectomy was performed in 11 patients; two patients were not candidates for surgery (nos. 2 and 9), and a secondary curettage was performed.

Tumours were graded according to Thiery & Willighagen (20): Grade I. neoplasms composed of gland-like structures, with either acinar or papillary arrangements. Grade II. less highly differentiated neoplasms in which both solid portions and glandular structures are recognizable. Grade III. tumours composed entirely of solid strands of epithelial cells without glandular structures. The number of tumours of grades I, II, and III is 6, 5 and 2, respectively (Table I).

In all patients the tissue specimens were prepared for paraffin sections and stained with haematoxylin and eosin. In 12 patients histochemical examinations were performed. In 2 patients (nos. 7 and 11) no carcinoma was found in the endometrium after the hormone treatment, only 10 cases were therefore available for comparative study of the endometrium before and after the treatment. Tissue for histochemical examinations was immediately quick-frozen with carbon dioxide, serially cut at 10 μ on a cryostat microtome and treated to demonstrate the activity of alkaline and acid phosphatase (lead and coupling techniques), and adenosine triphosphatase (9). Paraffin sections were also stained with PAS to demonstrate secretory activity (15). Diastase treatment of the sections did not influence the reaction.

Mouse liver and kidney tissue, prepared in the same way as the tumour specimens, served as controls for the demonstration of alkaline and acid phosphatase activity.

nges were seen. In one case more anaplastic ...e appeared after the treatment (no. 1).

...n tumours of Grade II histological changes ...re recorded in 2 out of 5 cases: in one case ...nthomatous areas (Fig. 7) were seen and in ...e other the tumour was registered as Grade I ...ter the treatment (no. 8).

In one of the 2 cases of Grade III glandular ...ructures and necrotic areas were seen after the ...reatment.

Histochemistry

The activity of alkaline and acid phosphatase showed a great ariability as revealed by the intensity and the localization of the staining. The activity could vary from strong to none in different localizations showing the same grade of differentiation (Figs. 6 and 7).

Alkaline phosphatase. In general, the enzyme activity was mostly localized toward the apical cell border and most frequently in the highly differentiated areas (Figs 3 and 5). I one case of Grade III no activity was found.

After the treatment the activity had decreased in 6 out of 10 cases. In one case the activity seemed unchanged, and in 3 cases enzyme activity was increased (Figs. 4 and 6).

Acid phosphatase. Enzyme activity was most pronounced in anaplastic tumour tissue. The activity was often present as intracellular granules (Fig 9). When enzyme activity was seen in highly differentiated tumour tissue this activity was present both toward the apical and the basal cell border (Fig 8).

After the treatment the activity had increased in 3 out of the 10 cases, in the others the activity seemed unchanged. In areas of necrotic tissue a pronounced activity was seen.



Fig. 4. The same tumour as in Fig. 3 after treatment. A more uniform alkaline phosphatase activity toward the apical cell border. Coupling technique, 190.



Fig. 3. Tumour of grade I. Alkaline phosphatase activity in scattered areas before treatment. Coupling technique, 190.

Table I *Clinical and therapeutic data*

Case no.	Age	Grade	Drug[a]	Total dose (mg)
1	55	I	PD	1 000
2	82	I	D	4 200
3	62	I	D	4 200
4	45	I	D	4 200
5	59	I	D	4 200
6	49	I	D	4 200
7	75	II	PD	21 000
8	59	II	D	4 200
9	66	II	D	4 200
10	58	II	D	4 200
11	53	II	D	4 200
1	60	III	PD	1 000
13	70	III	D	4 200

PD 17alpha hydroxy-progesterone-caproate. D 17alpha hydroxy 19-nor-progesterone-caproate.

Before and after treatment haematological studies, liver and kidney function tests, serum proteins, and serum electrolytes were performed.

In all patients luteinizing hormone (LH) in the serum

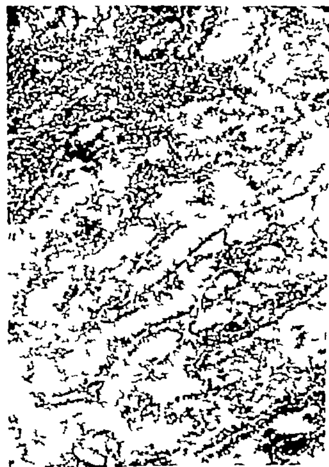


Fig 1 Tumour of grade I before treatment th progestogen. Only small amounts of PAS-positive material are seen. PAS, 190.



Fig The same tumour as in Fig. 1 after treatment. Great amounts of PAS-positive material are seen in the gland-like structures. PAS, × 190.

(14) and the 4-hour xcretion of oestriol in the urine (5) were determined before the treatment and eekly as long as the patients stayed in the hospital. I addition the 4-hour excretion of pregnandiol in the urine (14) and corticoids in the plasma (4 17) ere determined in 7 patients.

The patients were weighed weekly

RESULTS

Histology

The sections often revealed a great variation of the histological picture. The curettage specimen usually represents limited and superficial areas of the tumour. Therefore, only marked morphological changes after the treatment were recorded.

In general histological changes after the treatment were inconspicuous. The most convincing changes were recorded in tumours of Grade I in 4 out of 6 cases signs of secretory activity appeared (Figs. 1 and 2). In cases acanthomatous

nally presented low LH alues no changes were obtained.

The excretion of oestriol showed a arying course but in general no definite pattern was found (Fig. 12)

The excretion of pregnandiol and the concentration of corticoids in the plasma showed no definitive changes during the treatment.

Weighing esults

No weight changes occurred during the treatment.

DISCUSSION

The differentiated carcinomas seemed to give the most marked histological response to intense progestogen treatment of 3 weeks duration, iz. secretory and acanthomatous changes. Necrosis of tumour tissue decidualisation and increase of the stroma as described by others (1 11) were



Fig From another localisation in the same section as show in Fig 4. Mostly negative staining reaction. Acanthomatous plaque to lower right. Coupling technique 190



Fig 4. Tumor of grade I. Activity of acid phosphatase after treatment. Coupling technique, 190

not observed. Varga & Henriksen (22) claimed that the histological alterations were usually recognisable by the end of 2 weeks' treatment. If no changes were see within 4 weeks, the tumour was considered histologically non responsive. Sherman (17) stated that 75% of the tumours responded w thin 4 weeks and 25% after 8–1 weeks. Thus, it is possible that treatment of a longer duration than 3 weeks would ha e gi en a more marked response.

Earlier it has been postulated that well dif ferentiated endometrial carcinomas are more likely to respond to progestogens than those which are poorly differentiated (7 20), and Varga & Henriksen (22) stated that anaplastic tumours are refractory. In our series however one case of anaplastic carcinoma showed clear histological alterations.

Alkaline phosphatase activity showed great ariability. Generally the activity was most regularly found in morphologically well differen-



Fig 5 Tumour of grade II. Variable alkaline phosphatase activity most of the activity in gland-like structures. Coupling technique 190.

Adenosine triphosphatase Before the treatment enzyme activity was sporadically seen toward the apical cell border in highly differentiated areas (Fig. 10) After the treatment the activity mostly seemed unchanged.

Laboratory tests

In 4 cases an increase of ESR took place during the treatment in 3 cases a decrease in haematocrit values was seen. In one case LDH increased (257–955) during the treatment, and in another case a slight increase of SGOT (29–37) and SGPT (17–48) was found. In these _ latter cases the serum electrophoresis changed as serum albumin decreased and alpha-globulins increased during the treatment. Other laboratory tests were unchanged in all cases.

Special case report

Case no. 12: She had diabetes and asthma. Thymol tests before and after the treatment th 17alpha-hydroxy progesterone-caproate suggested chronic liver disease with normal bilirubin and transaminase values. For the curettage, and the radical hysterectomy 4 weeks later, halothane anaesthesia was used. Three days after the second operation the patient became icteric, on the fourth postoperati e day she had a dehiscence of the wound and again was given halothane anaesthesia for the re-suture. After this she developed increasing jaundice and transaminase values. The patient improved, but needed another operation one month later for a second wound rupture. Halothane was not used, and a liver biopsy taken during surgery showed biliary cirrhosis. When the patient died 11 days later in hepatic coma, the liver showed a massive acute necrosis. This case has been discussed elsewhere (19) an evaluation is difficult as she was xposed to the treatment with 17alpha-hydroxyprogesterone-caproate and three times to general anaesthesia with halothane

Hormone analyses

There was a decrease of the mean serum levels of LH during the treatment in the 11 postmenopausal patients (Fig. 11) In 2 cases which origi-



Fig 6 The same tumour Fig. 5 after treatment. Alkaline phosphatase activity in both gland-like structures and solid cell portions. Coupling technique 190

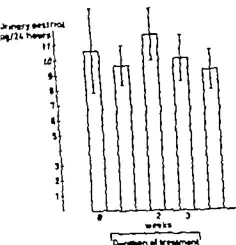


Fig. 1. The mean urinary oestriol levels in 13 women with endometrial carcinoma given progestogen treatment. Vertical lines represent ±S.E.

long-continued treatment, a pattern known to occur in human endometrium during the secretory phase (13).

The activity of acid phosphatase in human endometrium is most likely dependent on progesterone (6, 13). In the present study the progestogens did not seem to have e con incing effect upon tumour tissue of the endometrium. These findings are in agreement with Vokaer et al. (23) whereas Thiery & Willighagen (20) found a increase in acid phosphatase activity during progestational treatment.

In conclusion, intensive progestogen therapy of 3 week duration gives histological and histochemical alteration of primary adenocarcinoma of the endometrium. The changes are only moderate, nd differentiated carcinomas are more likely to respond than anaplastic ones.

The present study indicates that progestogens depress the serum levels of luteinizing hormone (LH) in patient originally presenting elevated LH alues. This is in agreement with Sherman & Woolf (18) who found the same pattern concerning urinary levels of LH after the administration of 17alpha-hydroxy-progesterone-caproate in patients with endometrial carcinoma. In postmenopausal women with high serum levels of LH treatment with chlormadinoneacetate also resulted in a depression (3).

The e cretion of oestriol did not show any definitive change during the treatment. Neither were any significant alterations found in the excretion of pregnandiol or the concentration of corticoids in the plasma.

Thus, 17alpha-hydroxy-progesterone-caproate and 17alpha-hydroxy 19-nor-progesterone-caproate seem to have an antigonadotrophic effect, whereas the adrenocorticotrophic function is probably unaffected.

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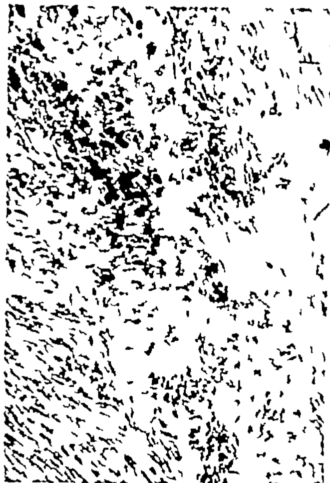


Fig 9 Tumour of grade II Intracellular acid phosphatase activity after treatment. Lead technique, 190.



Fig 10 Tumour of grade I. Mostly negative ATP-ase activity in tumour tissue. Strong positive reaction in vessel walls. 190.

tiated areas. This pattern is also demonstrated by other investigators (10 23) whereas Pfleiderer (16) found the activity of alkaline phosphatase most pronounced in clinically active tumours.

Acid phosphatase activity also showed the great variability as described earlier (23) but contrary to alkaline phosphatase the activity was most pronounced in undifferentiated tumour tissue. The very weak or absent activity of adenosine triphosphatase is in agreement with similar studies (16 20 23). Vokaer et al (23) stated that this absence of activity is characteristic of malignant change.

In some of the cases the hormone treatment was accompanied by changes in enzyme content of the tumour tissue especially pertaining to the alkaline phosphatase which often decreased during treatment. A similar pattern is also shown by Vokaer et al. (23) Thiery & Willighagen (20) however found that an initial increase of alkaline phosphatases was followed by a decrease after

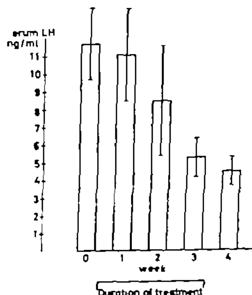


Fig 11 The mean serum LH levels in 11 postmenopausal women with endometrial carcinoma given progestogen treatment. Vertical lines represent ± S E.

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Among the CHC patients, 30 died of generalized disease and one from postoperative complications. Eighteen patients (36%)—10 of them with metastases—recovered. Four of these patients have been observed for 2–5 years and 14 for more than 5 years. Finally one patient has still active disease.

Forty-four patients suffering from IM (18) or OTP (26) were cured of their disease, including 5 with metastases. Determination of chorionic gonadotrophin was performed in 66 of the 94 patients at the time of the original diagnosis. Based on the findings at the re-evaluation, the frequencies of IM and CHC were one case per 126 000 and 49 600 births, respectively. The ratio of IM to CHC was 1 to 2.6. As compared with Norway and Sweden, Denmark seems to have the lowest incidence of these diseases. During the years 1943–1963 CHC accounted for approx. 0.03% of all malignant neoplasms in Danish females.

The study reported here forms the basis of subsequent immunologic investigations into the transplantation aspects of CHC and IM.

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In order to throw light on the occurrence of choriocarcinoma in Denmark all cases of the disease diagnosed in this country from 1940–1969 inclusive, have been subjected to a re-examination using a uniform evaluation and the histologic criteria accepted during recent years (3). The accomplishment of this work forms the basis of subsequent investigations into the immunologic conditions of this implantation tumour.

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Abbreviations

The following abbreviations are used in the tables and the text: CHC = choriocarcinoma or chorionepithelioma. IM = invasive mole or chorioadenoma destruens. OTP = other trophoblastic proliferations, which in the present work include the following diagnoses: hydatidiform mole, syncytial endometritis, placental polypus, molar residue abortion residue and unclassified cases.

Definitions

Following the generally accepted rules of today the criteria for the diagnosis of CHC are briefly as follows: An invasively growing, hemorrhagic tumour consisting of malignant trophoblast *with* *ut* the occurrence of chorionic illi. Often, the tumour tissue has a plexiform mode of growth with bars of cytotrophoblast covered with festoons of syncytiotrophoblast. Both types of cells must *alway be present.*

In the case of IM as the name suggests, one or more chorionic illi showing hydatidiform sw lling are observed, which—covered by proliferating trophoblastic tissue—invade the myometrium. This trophoblastic disease occurs only after (or simultaneously with) a simple hydatidiform mole, whereas CHC may occur after any pregnancy irrespectiv of its course

MATERIAL AND METHODS

From 1940 to 1969 Danish pathologists diagnosed 94 cases of CHC. However during this period, the classification of trophoblastic diseases has undergone considerable changes. This fact and the large number of various investigators prompted us to make a re-examination to ensure a uniform evaluation of the cases according to recent criteria of classification. The series under consideration was classified according to criteria laid down by the Armed Forces Institute of Pathology (3).

Our evaluation of the series was in all cases based on the pathologists original, histologic descriptions and diagnoses, and in almost half of the cases the original, histologic specimens or new sections from the original paraffin blocks were vailable Complete case records of all patients were at our disposal, and so were the results of pregnancy tests from most of the patients. Autopsy had been performed in 13 cases, in which the autopsy records ha also been in hand.

The original histopathologic diagnoses were divided into two groups. CHC and CHC? the question mark indicating that the pathologist had made his diagnosis with some reserv tion, e g strongly suggestive of CHC, probably CHC, etc. From these two groups of diagnoses the group of CHC? was excluded after the reappraisal of the series, which, on the other hand, resulted in the setting up of two new groups: IM and OTP The last-mentioned group included the cases which could not be grouped either as CHC or IM.

RESULTS

On the basis of histologic specimens 44 cases were investigated (Table I) They had originally been classified in 32 cases as CHC and in 1_ cases as CHC? In the re-examination of the first group the diagnosis was confirmed in 25 cases, whereas in 7 cases it was changed to IM Among the 1? cases originally suspected to be CHC, were diagnosed as CHC, 2 as IM whereas 8 tumours did not fulfil the criteria for any of these two groups.

In 50 cases (Table II) histologic material was not available and, therefore, the re-evaluation was based only on the original histologic descriptions and diagnoses. Among 41 cases originally diagnosed as CHC the diagnosis was confirmed in 1 after reappraisal of the descriptions. Seven cases were evaluated as IM and in 13 the descriptions gave no basis for classifying the tumours in any of these groups. Nine cases originally diagnosed with some reservation (CHC?) were classified as follows. CHC, 2 IM 2 and OTP 5

Of 26 cases which could not be grouped either as CHC or IM (Table III), hydatidiform mole was diagnosed in 10, syncytial endometritis in 4 and, finally 3 cases each were assigned to the following groups placental polypus, residue of hydatidiform mole, residue of abortion and unclassified.

Of 50 patients, with a diagnosis of CHC after re-evaluation (Table IV) 41 had metastases in the course of their disease Thirty of these

Table I *Classification of 44 cases of CHC before and after histologic reappraisal*

Original examination		CHC	IM	OTP
CHC	3	25	7	0
CHC ?	1	2		8
Total	44	7	9	8

Table II *Classification of 50 cases of CHC before and after reappraisal of the original descriptions and diagnoses*

Histologic specimens were not ailable

Original examination		CHC	IM	OTP
CHC	41	_1	7	13
CHC	9	2		5
Total	50	23	9	18

Table III *Classification of 26 trophoblastic proliferations which could not be grouped as either CHC or IM*

Histologic specimens were available in 8 cases

Diagnoses after reappraisal	Histologic specimens Yes	No	Total
Hydatidiform mole	4	6	10
Syncytial endometritis	2	2	4
Placental polypus	0	3	3
Residue of mole	0	3	3
Residue of abortion	2	1	3
Unclassified	0	3	3
Total	8	18	26

patients had died of generalized disease at the time of the conclusion of this study (November 1969) 10 patients had no symptoms of their previous disease and one had still active disease. In 9 patients, the disease was localized. One of these patients died from postoperative complications following hysterectomy whereas 8 had no symptoms of their previous disease. The periods of observation for the patients who were alive and well were 2–5 years in 4 and more than 5 years in the remaining 14 cases.

All 44 patients grouped as IM and OTP survived the trophoblastic disease. In 4 cases of IM and in one among the 26 cases of OTP metastases were observed. In the latter case, no histologic specimens were available, and it was impossible to decide whether the primary disease was CHC or placenta accreta (unclassified).

Autopsy was performed in 13 of the 30 patients who died of generalized CHC. In this group (Table V) metastases were revealed in 39 organs, i.e. an average of 3.0 afflicted organs per autopsy.

Table IV *Follow-up results of 94 cases of trophoblastic lesions reclassified on re-evaluation*

Diagnoses		Metastases No		Dead No		Alive No	
CHC	50	41	82	31	62	19[b]	38
IM	18	4	22	0	—	18	100
OTP	26	1	4	0	—	26	100
Total	94	46	—	31	—	63	—

One patient died from postoperative complications
One patient has still active disease at the present time

Table V *Localization of metastases in 30 cases of generalized, fatal CHC*

Organs	No. of cases Autopsy (13 patients)	No autopsy (17 patients)
Lung	11	11
Liver	7	0
Pelvic wall	2	3
Vagina	1	4
Brain	1	3
Kidney	4	0
Intestine	3	0
Lymph node	3	0
Spleen	2	0
Eye	0	2
Broad ligament	1	0
Ovary	0	1
Cervix uteri	1	0
Skin	1	0
Urethra	1	0
Omentum	1	1
Skeletal muscle	0	2
Total no. of affected organs	39	27
Average	3.0	1.6

Autopsy was not performed in 17 cases and—as might be expected—on an average, metastases were revealed in only about half as many organs as in the former group. A characteristic feature of the two groups is that metastases were most frequently found in the lungs.

Table VI shows the localization of metastases in 10 CHC patients who were considered to be cured of their generalized disease. It is noteworthy that the average number of organs showing metastases was of the same order as in the 17 fatal

Table VI *Localization of metastases in 15 cases of trophoblastic lesions*

Organs	No. of cases CHC (10 patients)	IM (4 patients)	OTP (1 patient)
Lung	7	1	1
Vagina	6	3	0
Spinal cord	2	0	0
Pelvic wall	1	1	0
Bladder	1	0	0
Lymph node	1	0	0
Total no. of affected organs	18	4	1
Average	1.8	1.0	1.0

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Table VII. *Results of pregnancy tests in the 94 patients*

The cases are classified according to the results of the re-evaluation

		No. of tests		
Diagnosis	No.	Pos.	Neg.	Not invest.
CHC	50	44	0	6
IM	18	18	0	0
OTP	26	23	3	0
Total	94	85	3	6

cases not subjected to autopsy and that the lungs in the group of survivors also had the highest frequency of metastases. In addition metastases were revealed in one organ of each of 4 patients with IM and one with OTP

At autopsy of 4 CHC patients who died of generalized disease the uterus revealed no trace of the primary tumour and in one patient—now without symptoms—who had hysterectomy performed after a pneumonectomy because of metastases from CHC the myometrium did not reveal any trophoblastic tissue. Moreover one patient who had been treated for metastases of one lung and the spinal cord 8 years previously was still well Hysterectomy was not performed in this case.

Finally in considering the metastatic spread of these tumours, it should be mentioned that bone metastases were not observed in any of the 46 patients (CHC IM, and OTP) who had trophoblastic tissue outside the uterus.

Based on the case records, a survey was made of the results of pregnancy tests carried out about the time of the histologic diagnosis (Table VII) The analyses extended over a period of 30 years and were carried out in different laboratories owing to variations in the test methods and their sensitivity and in the calculation and specification of the hormone content, the results are given qualitatively A positive reaction means that the laboratory had found such a large quantity of chorionic gonadotrophin in a specimen of urine that it was taken to be a definitely positive pregnancy reaction. However in all patients normal pregnancy could be excluded at the time of examination and the positive reaction should therefore be interpreted as an abnormal presence of active trophoblastic tissue either in or outside the uterus.

About the time when the trophoblastic disease was diagnosed histologically positive pregnancy reactions were found in 85 of the 94 patients. The pregnancy tests were negative in 3 cases, and no tests were performed in 6 cases, which were all classified as CHC at the reappraisal.

DISCUSSION

As compared with the original figures, this retrospective study has reduced the CHC cases by almost 50% as the diagnosis could be confirmed or rendered probable only in 50 of the original 94 cases.

In 50 cases, the reappraisal was based exclusively on the original histologic descriptions, and the value of a critical review on this basis may appear problematic. In the re-evaluation of the 44 cases in which histologic specimens were available, the agreement between the original descriptions and our own observations were, however so pronounced that it was found both practicable and justified also to revise the cases in which no histologic specimens were available

In the cases which were examined on the basis of histologic specimens, the diagnosis was confirmed in 61% whereas only 46% of the diagnoses could be accepted among the cases which were examined only on the basis of the descriptions. As a possible explanation of this difference, it should be mentioned that the two groups also differ as to the time of the diagnosis in the individual cases the former group containing far more cases from recent years. Thus, about three quarters of the former group date from 1955–1969 whereas only about one third of the latter group are from this period It is only reasonable that histologic specimens were less frequently available for re-examination in the cases diagnosed early in the period under consideration and it was often necessary to reclassify such cases. The cause of the reclassification of the many early cases should presumably be sought mainly in the fact that they preceded the publication of the criteria of classification used by us, or that these criteria had not yet been generally accepted. On the other hand, during the 30-year period under investigation all the original descriptions of the specimens

are characterized by a remarkable uniformity both as regards style and content.

In the re-evaluation 18 cases were diagnosed as IM, which in two respects differ from CHC: IM occurs only after (or simultaneously with) a hydatidiform mole, and it contains one or more villous structures. Some of the diagnoses were, as in the case of the CHC diagnoses, made before the present criteria of classification were accepted in this country whereas in other cases it is likely that villi were not present in the specimens used for the original examination. Judging from clinical experience, IM is rarely transformed into CHC, but it is evident from several histologic descriptions that some of the pathologists had had this possibility in mind. It is not unusual in records to find the following conclusion. IM with transition to CHC Diagnosis: CHC (IM?).

The differential diagnosis between CHC and IM is a prerequisite for the estimation whether the two tumours have the same or different prognoses. Both of these trophoblastic tumours grow invasively and are able to metastasize, and thus they fulfil the two fundamental conditions required in order to group a proliferative process under malignant neoplasms. Nevertheless, some investigations show (5, 8) that in patients with IM the prognosis is favourable, as contrasted with cases of CHC for which the prognosis has so far always been doubtful. This experience was confirmed by the present series, in which all IM patients including 4 with generalized malignancy were cured of their disease, but why the prognoses of these two tumours differ in spite of extensive parallels in their clinical biology remains an open question.

In 76 histologic descriptions we did not find sufficient evidence for grouping the cases under the diagnosis CHC or IM. On the contrary 23 of these descriptions, of which 8 were accompanied by relevant histologic specimens, contained so many characteristics deviating from these two diagnoses and indicating other trophoblastic proliferations that it seemed reasonable to group them under five different diagnoses. Only in three cases was it impossible to make any diagnosis from the descriptions of the specimens. The three patients concerned may have had CHC but in each of them one or more of the following diagnoses could not be excluded: residues from normal pregnancy or hydatidiform mole, placenta accreta, and syncytial endometritis.

Previously CHC was considered a highly malignant disease, but the introduction of cancer chemotherapeutic agents (6) in the treatment of the disease has to some extent changed this concept. In the light of this, a survival ratio of 36% in the present series may seem remarkable, especially when 14 of the 18 patients survived for more than 5 years without any symptoms of recurrence. We had this in mind in the reappraisal of all the cases, but we did not find any basis for a revision of these diagnoses.

It is a well-known fact that sometimes the primary tumour cannot be demonstrated in the uterus of patients with generalized CHC: this was observed in six cases in the present study. As possible explanations of this fact may be mentioned that the primary tumour may have regressed, or it may have been carried away in toto at a time when it was still relatively small. A third possibility is that the primary tumour was never present in the myometrium, but that the disease occurred when one or a few of the trophoblasts normally found in the lung tissue of pregnant women (1) turned malignant. In the last-mentioned case the primary tumour will often be misinterpreted as a metastasis. The problem is mentioned here in order to point out that these cases—beyond giving rise to theoretical considerations—constitute a special diagnostic problem, as the histories of these patients usually reveal nothing which could turn the pathologist's attention to trophoblastic neoplasm.

One more patient died of generalized cancer without any symptoms of gynaecologic disease having been observed during the preceding two months' hospital story. A previously extirpated lung tumour had been diagnosed as squamous-cell bronchogenic carcinoma, and a tumour which was later removed from the wall of the small intestine was interpreted as a metastasis from the lung carcinoma. Autopsy revealed a tumour measuring $6 \times 5 \times 5$ cm, in the myometrium, which on histologic examination proved to be a CHC. This diagnosis was confirmed on reappraisal of the specimens of the tumours previously removed.

The absence of gynaecologic symptoms thus tends to exclude a trophoblastic neoplasm. On the other hand, if bone metastases occur in patients in whom trophoblastic tumour has been diagnosed, this diagnosis should be reconsidered. It is a fact that CHC metastasizes very rarely to the

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osseous system, and the ovary is also only in exceptional cases the site of metastases.

Both normal and malignant trophoblasts synthesize chorionic gonadotrophin which can be measured e.g. in the urine by biological or immunological methods. Therefore, it is imperative to determine the presence of this hormone in patients in whom trophoblastic disease has been diagnosed. In 9 cases of the present series, the histologic diagnoses were not supported by the hormone assays, and there is thus a theoretical possibility that trophoblastic disease had erroneously been diagnosed in these patients. In 6 of them in whom the original CHC diagnosis had been confirmed by the reappraisal, no hormone determinations at all had been carried out. In 2, the diagnosis was not established until autopsy and 3 patients survived for only 6, 8 and 17 days, respectively, after the diagnosis had been made. Only in one patient, who died about 3 months after the disease had been observed, (in 1944), was there no reason why hormone analysis had not been carried out. On the basis of the histories of these patients, the objective findings and the histologic descriptions, which, in three cases, were supplemented with relevant histologic specimens and descriptions of autopsies, we have no reason to doubt the correctness of the original diagnoses.

In 3 cases, which were all classified as OTP on reappraisal, the results of the hormone analyses were negative, but also in these patients everything seemed to indicate that they had trophoblastic proliferations. In these cases the discrepancy between the result of the histologic examination and the hormone analysis may be explained either by the use of a not very sensitive method to demonstrate the hormone or by relatively long time interval between the removal of the hormone-producing tissue and the urinalysis.

Conception is a prerequisite for the conditions necessary for the development of gestational CHC and IM, and from a theoretical view it would be reasonable to mention the frequency of these diseases in proportion to the total number of conceptions. The total number of conceptions includes, however, several figures whose accuracy varies within wide limits. While the number of births is stated with great accuracy in many countries, there is generally some uncertainty as to the number of legal and, particularly, illegal abortions, and to this must be added the problem of the number of the non verified abortions. Consequently it must be reasonable to state the incidence of CHC and IM in absolute figures for a given period of time and also to consider the relative frequency of the diseases in relation to the birth rate. But it should be borne in mind that the birth rate represents different shares of the total number of conceptions, depending on the existing abortion and contraception legislation in the various countries. Furthermore, it would be of interest to know how many cases of CHC are included in the total number of malignant neoplasms in the various countries.

During the 30-year period covered by this study 49 cases[1] of CHC were diagnosed, and in the same period the average annual birth rate was about 80 000. Thus, one case of CHC per 49 000 births was registered. In comparison, it may be mentioned that the incidence of CHC in Norway (5) has been found to be one case per 6 000 births in the period from 1953 to 1961 whereas one case per 41 000 births and abortions was registered in Sweden from 1958 to 1965 (8).

From 1940 to 1969 another 19 cases of IM were diagnosed in Denmark, i.e. one case per 126 000 births, and the ratio of IM to CHC was thus found to be 1 to 2.6. In Norway (5) the ratio of the two diseases was 1 to 3.5 from 1953 to 1961 and in Sweden 1 to 1.9 (1958–65) (8).

From 1943 to 1963 inclusive about 130 000 cases of malignant neoplasm were registered in Danish women (2). In the present investigation, 43 cases of CHC were diagnosed during the same period. This number corresponds to 0.03 % of all cancers in females diagnosed during the years in question.

The numerous papers published on malignant tumours originating from the fetal placenta seem to be somewhat out of proportion to the rare occurrence of these diseases. The reason for publishing an additional paper on this subject must be sought in our interest in investigating whether the growth and metastatic spread of these tumours are the same whether tumour and patient have identical or different tissue types (HL-A and ABO types). In order to investigate these immuno-

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One case has been excluded because the patient was a foreign citizen.

[1] This investigation revealed 18 cases, and one case was reported to the Cancer Registry as IM. This diagnosis was confirmed by re-examination.

logic problems it is strictly necessary that only patients who have or have had CHC or IM are involved in the investigations

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PHOSPHOLIPID COMPOSITION OF HUMAN AMNIOTIC FLUID DURING GESTATION AND AT TERM

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Abstract. The concentration, composition and fatty acid components of amniotic fluid phospholipids were determined in samples obtained at term and during the second trimester of gestation. The mean concentration of total phospholipids was significantly higher at term than during the second trimester. There was also a highly significant increase in the ratio phosphatidylcholine/sphingomyelin at term. Concomitantly the proportion of palmitic acid in the phosphatidylcholines rose to very high levels. Changes in the same direction were recorded in the fatty acid composition of the phosphatidylethanolamines. The present results indicate that analysis of amniotic fluid phospholipids may be of clinical significance in determining the pulmonary maturity of the foetus.

The presence of fully saturated phosphatidylcholine and other phospholipids in human amniotic fluid was qualitatively demonstrated in 196_ by Helmy & Hack (8). Quantitative analysis was later carried out by Biezenski et al. (4) and by Nelson (10). However these studies did not include fatty acid analysis of the phospholipid fractions. The fatty acid structure of amniotic fluid phospholipids, and particularly of the phosphatidylcholines, is of special interest in view of the evidence that the amniotic fluid phospholipids originate from the lungs of the foetus (11). The idiopathic respiratory distress syndrome (IRDS) is closely linked to deficiency in alveolar dipalmitoyl-phosphatidylcholine (1, 2, 6) and therefore a detailed examination of the amniotic fluid phospholipids might be of diagnostic aid in assessing the maturity of the foetal lungs, as previously suggested by Scarpelli (17). To evaluate this possibility we have determined the phospholipid composition and the fatty acid structure of phosphatidylcholines and phosphatidylethanolamines of amniotic fluid during gestation and at term. While this work was in progress Gluck et al. (9) reported changes in the mean concentration of sphingomyelin and phosphatidylcholines in amniotic fluid during pregnancy.

MATERIALS AND METHODS

Amniotic fluid was obtained from 16 women at term and from 23 pregnant women in the 13th to 24th week of gestation. In the latter group the pregnancy was interrupted by legal abortion on socio-medical grounds and the women were all healthy. The samples of amniotic fluid obtained in this group were characterized by the crown-heel (CH)-lengths of the foetuses (Table I) since experience shows that information about the duration of pregnancy is unreliable, especially from women with large foetuses. None women in the term pregnancy group showed symptoms of hepatosis (itching and serum glutamate-pyruvate transaminase >100 units). The newborn infants developed normally in all 16 cases.

Samples of amniotic fluid from term pregnancies were taken at amnioscopy. The pole of the amniotic sac was visualized and punctured. Amniotic fluid was collected directly from the cannula into centrifuge tubes. In the abortion group the samples were collected at surgical termination of the pregnancy. Abdominal hysterotomy was performed and the intact amniotic sac was removed and punctured. Thus the samples in both groups were obtained by direct puncture of the amniotic sac which eliminated contamination by blood. All samples were immediately centrifuged at 1 000 g for 15 min. Floating debris was removed by filtration through paper. The samples were then frozen and stored at −20° until analysis, which was performed within 1 2 months.

Analytical methods. The lipids were extracted with chloroform methanol according to the principles of Bligh & Dyer (5) as follows. Each sample of amniotic fluid was transferred to a separating funnel, and 25 sample-

Table I *Distribution of amniotic fluid samples obtained at legal abortions*

CH-length of foetus (cm)	No of samples
11	1
12	1
14	1
15	5
16	1
18	1
20	3
21	5
22	2
24 (duplex)	1
26	1
30	1
Total	23

volumes of methanol + 1.25 sample volumes of chloroform were added. After shaking the funnel, another 1.25 sample-volumes of chloroform were added and the mixture was again shaken. Finally 1.25 sample volumes of 0.9% (w/v) NaCl in water were added and the funnel was left over night. Two clear phases were obtained. The lower chloroform-phase, containing the lipids, was taken to dryness *in vacuo* and the lipids were re-dissolved in a small volume of chlorof rm. Aliquots were taken fo determination of phosphorus. The remainder was used for fractionation of the phospholipids by ascending thin-layer chromatography on glass plates, 20 20 cm. The plates were coated with a 0.5 mm thick layer f Silica Gel H (Fluka AG Buch S.G). The developing solvent was chloroform/methanol/acetic acid/water 25 15 4 2 (v/) (Skipski et al 13). Prior t use the adsorbent layers were washed by running the plates in the developing solvent mixture until the solvent front had reached the upper edge of the plates. They were then activated at 120 for 1 h and subsequently stored in desiccators over P_2O_5. The samples were applied to the plates as bands, the length (1–4 cm) depending on the size of the sample

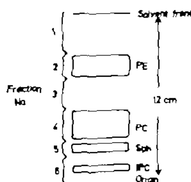


Fig 1 Identification of phospholipid fractions isolated by thin-layer chromatography of lipid xtracts from amniotic fluid. The position of authentic phospholipid standards is indicated. Abbreviations: PE, phosphatidylethanolamine PC, phosphatidylcholine Sph, sphingomyelin lPC, lysophosphatidylcholine

After development the plates were exposed to the vapours of iodine for visualization of the lipid fractions. The chromatogram of each sample was divided into six area These and corresponding blank areas were scraped o the plate into conical centrifuge tubes. To each tube added 2 ml of chloroform/methanol/acetic acid/ wa 50 39 1 10 (/v) which quantitatively extracted t phospholipids from the silica gel (3). The tubes e agitated in a mechanical tube-shaker and centrifug Aliquots of 700 μl were taken from the clear supe natant for phosphorus determination. The chroma graphic mobility of the six isolated fractions in relati to those of authentic phospholipid standards is shown i Fig. 1 Recovery of phosphorus from the thin-layer pla was 94.6% ± 9.1 (mean ± S.D for 32 samples). Bla values were reduced to near zero by pre running the thi layer plates in the developing solvent.

Phosphorus determinations were carried out in duplica by a slight modification of the procedure described b Chen et al. (7) Each sample i chloroform solution taken to dryness in a borosilicate glass test-tube an 3 drops of concentrated sulphuric acid were added. Th tubes were heated on a sandbath until hite fumes o sulphur trioxide appeared. To each tube were then adde 4 drops of 72% perchloric acid and the tubes we heated in a small Bunsen flame until the contents er quite clear Colour development was done directly i the test-tubes after the addition f 4 ml of 0.56 M NaOH followed by 4 ml ascorbic acid reagent of Chen et al (7). The tubes were heated at 65 for 5 min, then coole and the absorbance read at 820 nm.

The fatty acid composition of the phosphatidylcholine and phosphatidylethanolamine fractions was determine after transesterification of the phospholipid in ml o absolute methanol containing 2% (/v) conc. sulphuric acid and, as antioxidant, 0.05% (w v) 2,6-di-tert. butyl-p-cresol (British Drug Houses, London). Transesterification was complete after 3 hours at 65 and the resultant methyl esters were xtracted wi light petroleum ether (b p. 40–60°) after addition f water to the reaction mixture. The methyl esters were purified by thin-layer chromatography on Silica Gel G (E. Merck, Darmstadt) with hexane benzene 1 1 (v) as the moving phase. In this system the approximate R_f-value is 0.5 for long-chain fatty acid methyl esters. They were visualized under UV-light after the plates had been sprayed w th solution f 0.2% (w/) dichlor fluorescein (Fluka, Basel) in ethanol. The methylesters were quantitati ely luted ith a mixture of diethyl-ether petroleum ether 5 95 (v), and then analyzed by gas-liquid ch omatography an F & M 402 Gas Chromatograph equ pped th flame ionization detectors The stationary phase as 10 ethylene glycol succinate polyester on acid-washed Celite, 100–120 mesh. U-shaped glass columns, 6 ft 3 mm (internal diameter) ere used. Peak areas ere deter mined by an lectronic integrator (Hewlett Packard 3370 A Integrator)

RESULTS

All the samples obtained from the abortions were grouped together since there was no consistent

Table II *Concentration and composition of amniotic fluid phospholipids*

There were 16 samples in the term pregnancy group and 23 in the abortion group
Abbreviations: PL, phospholipid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; Sph, sphingomyelin

Gestational group		Total PL-P (mg%)	Phospholipid fraction (see Fig. 1) (% total P) 1	2 (PE)	3	4 (PC)	5 (Sph)	6	PC/Sph (moles/mole)
Term pregnancies	Mean	0.142	5.1	8.8	9.5	67.6	8.1	0.8	10.99
	S.D.	(±0.089)	(±1.7)	(±2.9)	(±2.7)	(±6.8)	(±3.9)	(±0.6)	(±6.51)
	Range	0.048–0.376	2.3–8.9	4.0–13.4	5.3–15.8	50.0–78.6	3.0–16.8	0.0–2.7	2.98–23.80
Abortions (foetal CH-length 11–30 cm)	Mean	0.074*	4.7	10.9	8.2	41.2*	33.9*	1.2	1.30*
	S.D.	(±0.035)	(±2.5)	(±3.2)	(±3.1)	(±7.8)	(±6.8)	(±0.8)	(±0.44)
	Range	0.028–0.195	1.5–12.5	7.4–16.2	3.0–13.5	28.8–51.1	24.8–45.8	0.0–3.7	0.63–2.50

* $p < 0.001$ for the difference between the means in the Student's t-test.

ariation in the analytical data with respect to the CH-length of the foetus. In 22 of the 23 samples the total concentration of phospholipid-P ranged between 0.028 and 0.118 mg%. In these samples the variation in the proportions of the two major phospholipids, phosphatidylcholine and sphingomyelin, was 29–51% and 25–46% respectively. One sample, which was obtained at a foetal CH-length of 11 cm, was exceptional in having a total concentration of 0.195 mg% phospholipid-P and a high proportion of phosphatidylcholine, 64.3%. The molar ratio phosphatidylcholine/sphingomyelin in this sample was 2.50, while in the other samples it varied between 0.63 and 2.00.

The total concentration of phospholipid-P in the 16 samples obtained from term pregnancies ranged between 0.048 and 0.376 mg%. In these samples phosphatidylcholine was always the major phospholipid, accounting for 50–79% of total phospholipid-P. Phosphatidylcholine/sphingomyelin ratios ranged between 2.98 and 23.80. The lowest ratio occurred in the sample with the lowest total concentration (0.048 mg%). This sample also had the lowest proportion of phosphatidylcholine, 50%. Mean values ± S.D. and ranges for the above parameters of the two groups are given in Table II.

Fatty acid analysis of the phosphatidylcholine fraction was carried out on 15 individual samples in the abortion group and on 13 obtained from term pregnancies, consisting of 10 individual samples and three obtained by pooling the phosphatidylcholine fractions from different samples of amniotic fluid. Palmitic acid was the dominant phosphatidylcholine fatty acid in both groups but the percentage levels were significantly different (Table III). In the abortion group it varied between 44 and 57 mole% of total fatty acids while the range at term was 65–84 mole%. There was also a higher level of palmitic acid in the phosphatidylethanolamine fraction (Table III) at term than earlier during gestation.

DISCUSSION

The identification of different phospholipid fractions was based solely on their chromatographic mobility in one solvent system and should therefore be regarded as tentative. However, it is clear that the phospholipid pattern of amniotic fluid at term is distinctly different from that during the second trimester of pregnancy. The observed high values at term of the ratio phosphatidylcholine/sphingomyelin is in agreement with previous investigations by Biezenski et al. (4) and by Nelson (10). Gluck et al. (9) recently reported that this increase took place abruptly around the 35th week of gestation. However, the normal range of variation of the phosphatidylcholine/sphingomyelin ratio was not given but the authors stated that inspection of the thin-layer chromatogram had proved sufficient to decide whether the phospholipid pattern was of the mature type or not. The wide range of ratios which we found at term in the present study points to the possible existence of borderline cases where it might be difficult to assess the degree of foetal lung

Table III *Fatty acid composition of amniotic fluid phosphatidylcholines and phosphatidyl thanolamines*

The fatty acids are designated by (umber of carbon atoms) (umber of double bonds). Phosphatidylcholine data are means ±S.D for 13 samples from term pregnancies and 15 samples obtained at legal abortions. Phosphatidylethanolamines ere analyzed on pooled samples from 4 term pregnancies and 5 pooled samples from legal abortions

Gestational group	Phospholipid		Fatty acid (mole %) 14 0	16 0	16 1	18 0	18 1	18	20 4
Term pregnancies	Phosphatidylcholines	Mean	5 1	77 1	4 9	4 4	7 1	1 4	—
		S.D	(±2.2)	(±6.8)	(±2.4)	(±1 0)	(±2.1)	(±1.2)	—
		Range	2.9–10.1	64 7–84 1	0 0–8.8	3.0–6.2	3.5–10.4	0 0–3.7	—
Abortions[b]	Phosphatidylcholines	Mean	2.0[a]	50.3[a]	2.3	20.8[a]	1.8[a]	2.9	—
		S.D	(±1.6)	(±4 1)	(±1 6)	(±4 1)	(±4.8)	(±2.4)	—
		Range	0.7–6.2	44.3–56.8	0 0–5 7	15 1 31 0	14 7 28.8	0.5–9.5	—
Term pregnancies	Phosphatidylethanolamines		1.2	36.4	5.6	12.7	32.9	3.6	7.6
Abortions[b]	Phosphatidylethanolamines		—	21.8	—	35 0	24.5	3.8	14.9

[a] $p<0.001$ for the difference between the means in the Student s t-test.
[b] Foetal CH-length 11 26 cm.

maturity on the basis of this ratio alone. This is illustrated by the complicated phospholipid pat terns in abnormal pregnancies reported by Nelson (10) Thus, in one of his cases (M D) where the infant (2 825 g) developed signs of IRDS but recovered, the ratio of phosphatidylcholine/ sphingomyelin in the amniotic fluid was 13.50 (calculated from Table IV in ref 10) but the percentage levels of sphingomyelin and phosphatidylcholine were both exceptionally low

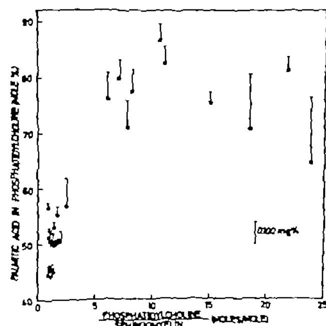


Fig. 2 Characterization f amniotic fluid phospholipids. Each point represents ne sample obtained either at term (■) or during gestation at foetal CH length of 11 26 cm (●) The concentration of phosphatidylch line-P is indicated by the ertical bars.

The fatty acid composition of the phosphatidylcholines may prove to be of equal or even greater diagnostic value than the phospholipid composition. All samples from term pregnancies in the present study had far higher proportions of palmitic acid in the phosphatidylcholines than the samples from the abortion group (Table III) This increase in palmitic acid was linked to a cor responding decrease in stearic and oleic acid The high level of palmitic acid at term supports earlier evidence (11) that the amniotic fluid phosphatidylcholines originate from the lungs of the foetus. There are no other reports on the fatty acid composition of amniotic fluid phospholipids. Gluck et al. (9) recently stated that amniotic fluid phosphatidylcholines had a fatty acid com position resembling that of lung effluents, gastric aspirates and meconium but no figures were given A highly saturated fraction of phosphatidylcholines from amniotic fluid and from lung effluents was found to contain more myristic acid at the 31st week of gestation than at term while our data show an increase in myristic acid at term (Table III where myristic acid is de signated as 14 0) This discrepancy may possibly be because

phatidylcholine fraction while the data of Gluck et al. refer to a subfraction obtained by precipitation in acetone.

The interrelationship between the molar ratio of phosphatidylcholine/sphingomyelin and the proportion of palmitic acid in phosphatidylcholine is depicted in Fig. 2. This figure also illustrates the wide variations in the absolute concentration of phosphatidylcholine in the amniotic fluid at term. The mapping of amniotic fluid phospholipids in a diagram such as that of Fig. 2 may be of twofold clinical significance. Firstly it may serve to help date foetal maturity. Secondly it may be of prognostic value in judging the risk that the newborn may develop IRDS. However this remains to be proved. Although Gluck et al. (9) stated that IRDS does not occur when the amniotic fluid phospholipid pattern is of the mature type more data are required in order to show the composition and fatty acid structure of amniotic fluid phospholipids at delivery in such cases where the newborn did develop IRDS. Apart from its possible clinical significance information on this point would also illuminate the current pathophysiological problem of IRDS, i.e. whether the syndrome is related to a prenatal deficiency of lung phospholipid surfactant (1, 2) or to a postnatal consumption and disappearance of surfactant originally present at birth (6).

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THE PREMENSTRUAL SYNDROME

Clinical Trial of Treatment with a Progestogen Combined with a Diuretic Compared with Both a Progestogen Alone and with a Placebo

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Abstract. T   series of clinical trials in the treatment of premenstrual tension are reported. I the first series, 35 patients hose ages ranged from 21 to 40 participated, representing total number of 140 menstrual cycles. The effect of progestogen-diuretic preparation was compared ith the effect of progestogen only. A good or very good effect as found in 26 and 17% of the cycles respectively. The difference as not statistically significant. In the second series 21 student nurses participated representing total number of 84 menstrual cycles. In this group the effect of the combined preparation as compared ith the effect of placebo in double blind series. The result as surprising, revealing no difference in effect in the two groups. Neither of the trials ga encouraging results. Other investigators have obtained far better results especially ith preparations containing tranquilliser in addition to progestogen-diuretics. A psychological factor apparently predominant in many of these patients.

The premenstrual syndrome is   complex of symptoms occurring in the latter half of the menstrual cycle and disappearing at onset of the menstrual flow. The time of occurrence, the severity of the symptoms, and their number may ry considerably. The syndrome as first described by Frank in 1931.

It has been maintained that premenstrual tension is the most common of the minor endocrinological diseases. Bickers & Woods (1951) found that among 1 500 female workers in a factory 36 asked for treatment for premenstrual complaints. Israel (1938) mentioned a frequency of bout 40 among women who otherwise ere healthy.

A number of theories ttempting to explain this syndrome ha been presented and the aetiology is not yet lea . Frank (1931) thought that the reason was to be found in the increased level of oestrogen in the body while Gillman (1942) and Hamblem (1945) asserted that progesterone was the hormone responsible. In most of the patients there seems to have been premenstrual oliguria and 1–2 kg increase of bodyweight. It is commonly thought that this fluid retention is connected with the increased production of steroids. It is well known that oestrogens may give rise to increased fluid retention in the tissues. Progesterone seems to be an aldosterone antagonist. A premenstrual deficit of progesterone may lead to increased aldosterone activity which, in turn, causes a tendency to oedema.

The syndrome includes a number of psychological as well as somatic symptoms. A definite positive connection has been found between the severity of symptoms and the psychological constitution of the patient (Rees, 1953). Neurotic subjects seem to ha e a greater tendency to such complaints (Coppen & Kessel, 1963). Frequently an obvious depressi mood prevails and also a change in the pattern of behaviour with increased irritability and irascibility. Dalton has shown (1959 1964) an increased tendency to accidents and criminal behaviour before and during menstruation. Admissions to hospital due to psychiatric complaints also increase t this time.

Among the somatic symptoms headache of migraine-lik character often occurs. Tense sore breasts, meteorism and abdominal pain, nausea and omiting may be found in quite number of patients and in varying degree. A feeling of tiredness has been recorded by many.

Many different treatments ha been tried. Because of the uncertain aetiology the treatment

Table I *Comparison of the effect of the combined preparation progestogen + diuretic and progestogen only in 35 patients representing a total number of 140 menstrual cycles*

	Progestogen + diuretic		Progestogen	
	No of cycles		No. of cycles	
No effect	4	34.3	36	51.4
Some effect	28	40.0	22	31.4
Good effect	14	70.0	11	15.8
Very good effect	4	5.7	1	1.4
Total	70	100	70	100

generally has been empirical and results obtained have been variable. This undoubtedly is due to the fact that the predominant symptom differs from patient to patient and that the treatment therefore must be individualised. Hoftun Knutsen (1966) maintained that the treatment may have 4 different aims: Influence of psychogenic factors, simple symptomatic treatment dehydration of the patient and neutralization of the dominant oestrogenic influence.

Any form of psychotherapy is valuable and necessary in most of the cases. If the patient will understand her complaints, she becomes less anxious and accepts her symptoms as a part of a natural function of her body Several need mild analgesics or tranquillizers in addition to this psychotherapy

Moreover most clinicians direct their treatment against fluid retention and a deficit of progester one A number of trails have been performed with a view to studying the efficiency of treat ment with diuretics either alone or combined with progestogen preparations (Greene & Dalton 1953 Rees, 1953 Swyer 1955 Dalton, 1959 Soule 1960 Warfield, 1961 Barfield Jungck & Greenblatt, 1962 Meyer 1963) The results obtained have been different and satisfactory improvement in 50–80 of patients has been reported. An especially good effect may be obtained by a combination of progestogen, diuretics and tranquillizers. Barfield et al. (1962) have reported a satisfactory effect in 24 of 31 patients. Meyer in 1963 indicated a good result in 71 and Warfield in 1961 calls the combined preparation of progestogen-diuretic tranquillizer (Cytran) as "most effective in relieving the symptoms of premenstrual tension" The complaints of the patients were as bad as ever after stopping the preparation and he found no difference depending on the patient s age or the duration of the symptoms.

MATERIAL AND METHODS

On the basis of the optimistic results which have been obtained by a combination of progestogen + diuretic and tranquillizers we were interested to compare the efficiency of progestogen + diuretic and progestogen only

Three doctors selected for the trial, from their practice, *patients suffering from typical premenstrual complaints*. A total of 35 patients aged from 1 to 40 years participated. Four bottles, numbered 1 t 4, each containing 30 tablets with an identical appearance were distributed to each patient. Two of the bottles contained the combined preparation medroxyprogesterone acetate 5 mg and *n*-penthyl-hydroxyflumethiazide mg, and two glasses contained 5 mg of medroxyprogesterone acetate only The contents of the tablets were unknown to the patients as well as to the doctor and the code was delivered from the manufacturer only after the trial was finished and the results were evaluated.

At the same time a schedule for each bottle as given to the patient on which she had to mark in details of her complaints and also the time in the menstrual cycle, at which these complaints occurred. The dosage was 1 tablet three times daily for the last 10 days before expected menstruation. For each day she marked the type and severity of her complaints.

The results of this series appear in Tables I–III here good or very good effects were found in only 17% of the cycles in the progestogen treated group. The results with the progestogen + diuretic treated group were better 26%, but the difference was not statistically significant at the 95 level. The effect on different symptoms as approximately the same in both groups (Table III).

Since the difference between these groups was hardly noticeable we found it worthwhile to perform a double

Table II *Type and incidence of premenstrual symptoms expressed in number of cycles and percent in 35 patients representing a total number of 140 menstrual cycles*

	Number	Percent
Psychological symptoms	113	80
Indisposition	11	80
Distended stomach	84	60
Oedema	48	34
Tense breasts	95	66
Abdominal pain	104	81
Headache	68	48
Nausea	—	15

Table III. *The effect of the combined preparation progestogen + diuretic (p + d) and of progestagen (p) on the various symptoms in 35 patients representing a total number of 140 menstrual cycles*

	No effect		Some effect		Good effect		Very good effect	
	p+d	p	p+d	p	p+d	p	p+d	p
Psychological symptoms	37	36	15	4	1	10	4	6
Indisposition	38	38	12	11	3	2	4	4
Distended stomach	28	27	7	9	3	3	4	3
Oedema	15	19	5	3	8	1	4	1
Tense breasts	27	27	6	7	5	5	9	9
Abdominal pain	40	25	7	14	4	2	8	4
Headache	27	28	5	3	0	0		3
Nausea	7	10	4	0	1	0	8	0
Total	219	210	41	51	17	23	35	30

blind trial with combined preparation and placebo. To find the most uniform group we asked the school of nursing at the hospital for their cooperation. Among 180 student nurses in the age range 20-22 years old 28% asked for treatment, but only about 20% suffered from real premenstrual complaints. A total of 21 students completed the trial. The same method was used as above. In the numbered bottles from 1-4, two contained the active preparation and two placebo, and all tablets were of the same appearance. The bottles containing active preparation were unknown to the subject as well as to the author until the trial was completed.

The results appear in Tables IV VI. The results are surprising since we found no difference between the two groups in which good or very good effects are recorded in 19 and 21 respectively. The effect on different symptoms did not differ essentially in the two groups.

DISCUSSION

The clinical trials did not reveal any differences in the results of treatment of the premenstrual syndrome with progestogen, with progestogen/diuretic combination or with a placebo. This seems to indicate that the observed effect of treatment of the premenstrual syndrome with progestogens or diuretics depends to a high degree on psychological factors. The success of treatment reported by Barfield et al. (1962), Meyer (1965) and Warfield (1961) compared with the results achieved in this series also points in the same direction. The preparation used by these authors contained a tranquillizer in addition to progestogen and diuretics.

It is, however, necessary to stress that the results are based upon the patients' subjective description of their symptoms, and this is not always reliable as a basis for recording and evaluating the efficiency of treatment.

However, the results seem to indicate that further research is necessary before progestogens

Table IV. *Comparison of the effect of progestogen-diuretic and placebo in 21 pupil nurses representing a total number of 84 menstrual cycles*

	Progestogen-diuretic No. of cycles		Placebo No. of cycles	
No effect	28	66.7	28	66.7
Some effect	5	11.9	6	14.3
Good effect	4	9.5	4	9.5
Very good effect	5	11.9	4	9.5
Total	42	100	42	100

Table V. *Type and incidence of premenstrual symptoms expressed in number of cycles and percent in 21 pupil nurses representing a total number of 84 menstrual cycles*

	Number	Percent
Psychological symptoms	59	70
Indisposition	66	79
Distended stomach	80	95
Oedema	5	6
Tense breasts	54	64
Abdominal pain	80	95
Headache	35	42
Nausea	10	12
Other complaints, itching, perspiration, fainting etc.	15	18

Table VI. *The effect of the combined preparation progestogen + diuretic (p + d) and of placebo (p) on the various symptoms in 21 pupil nurses representing a total number of 84 menstrual cycles*

	No effect		Some effect		Good effect		Very good effect	
	p+d	p	p+d	p	p+d	p	p+d	p
Psychological symptoms	19	22	5	2	1	4	4	2
Indisposition	22	24	5	2	3	4	4	2
Distended stomach	28	30	6	3	3	4	4	2
Oedema	3	2	0	0	0	0	0	0
Tense breasts	22	19	0	4	2	3	3	1
Abdominal pain	27	27	7	6	3	3	4	3
Headache	14	12	3	1	1	2	2	0
Nausea	4	4	1	0	1	0	0	0
Other complaints perspiration, fainting, itching etc.	8	4	0	1	0	1	0	1
Total	147	144	27	19	13	22	21	11

and diuretics are accepted as preparations to be prescribed routinely for the premenstrual syndrome.

ACKNOWLEDGEMENTS

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INACTIVATION OF OXYTOCIN BY HUMAN MYOMETRIAL PREPARATIONS

I. Preliminary Evidences for the Presence of a Thiol-Oxido-Reductase Activity and an Amino-Peptidase Activity in Crude Extracts

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Abstract. In a planned series of studies of the ways in which human myometrial preparations inactivate oxytocin a pilot study was made with the three synthetic model substrates L-Cystyl-di-β-Naphthylamide, S-Benzyl-L-Cysteinyl-β-Naphthylamide, S-Benzyl-β-Mercaptopropionyl-β-Naphthylamide and with Oxytocin and Desamino-oxytocin. The synthesis of S-benzyl-L-cysteinyl-β-naphthylamide and S-benzyl-β-mercaptopropionyl-β-naphthylamide are described. Incubations with crude extracts from myometria of non-pregnant women and pregnant women in the three trimesters liberated β-naphthylamine from L-cystyl-di-β-naphthylamide and S-benzyl-L-cysteinyl-β-naphthylamide but not from S-benzyl-β-mercaptopropionyl-β-naphthylamide. The liberation of β-naphthylamine from L-cystyl-di-β-naphthylamide was competitively inhibited by oxytocin and non-competitively inhibited by desamino-oxytocin. The results are interpreted as giving preliminary evidence for the presence of a thiol-oxido-reductase activity and an amino-peptidase activity in the extract.

Upon incubation in vitro with a variety of mammalian body fluids and tissue preparations oxytocin is inactivated, i.e. its typical biological effects disappear. In several instances the mechanism behind the inactivation has been studied. Thus oxytocin is inactivated by an enzyme present in plasma (or serum) of pregnant women. The enzyme, extensively purified by Tuppy & Wintersberger (13) and Sjöholm (10) is an amino-peptidase. It requires a free amino-group for its action. It catalyzes the rupture of the peptide bond between the cystine residue and the tyrosine residue of the intact oxytocin molecule. Oxytocin is also inactivated by liver slices and liver cell extracts (mouse, beef). This inactivation seems to involve first an enzymic, reductive cleavage of the disulphide bond by a thiol-oxido-reductase followed by an amino-peptidase activity (8). This amino-peptidase has been partially purified by Bartolek et al. (2). The enzyme requires a rupture of the disulphide linkage before it can act. In this respect it differs from the plasma enzyme of pregnant women. Recently the mechanism of inactivation of oxytocin by a partially purified rat kidney enzyme has been studied by Koida et al. (6). This enzyme attacks oxytocin at the peptide bridge between the leucine and glycinamide residues, releasing glycinamide.

The various known ways of enzymic attack of the oxytocin molecule by body fluids and tissue preparations are shown in Fig. 1. For a review see also Tuppy (11).

Myometrial preparations from human beings and other mammals inactivate oxytocin. However studies of the mechanism behind the inactivation have been made only with rat uterine preparations. Audrain & Clauser (1) reported that pieces of intact rat uterus inactivate oxytocin in the same way as that reported for liver preparations but that the inactivation took place only in an N_2-milieu and to a lesser extent in air but did not take place at all in an O_2-milieu. This is in contrast to the attack by the liver enzyme system which takes place both in N_2- and O_2-atmospheres. Glass et al. (5) reported that soluble and particulate fractions of homogenates from rat uteri inactivate oxytocin. They extensively purified an enzyme preparation from the soluble fraction

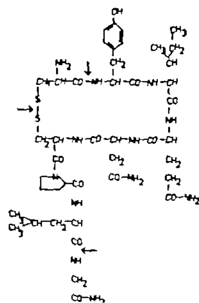


Fig 1 Known enzymic attacks of oxytocin by mammalian body fluids and tissue extracts (*arrows*). All leading to loss of biological activity

which acted in the same way as the enzyme preparation from rat kidney studied by Koida et al. (6)

This paper is the first in a planned series of studies of the ways in which human myometrial preparations inactivate oxytocin. It gives some preliminary information that crude human myometrial extracts contain a thiol-oxido-reductase activity and an amino-peptidase activity capable of attacking oxytocin.

PLAN OF THE STUDY

The purpose of this work was to get information about the inactivation of oxytocin by studying the degradation of well-characterised, synthetic model substrates instead of studying the degradation of oxytocin itself The model substrates used were L-Cystyl-di-β-Naphthylamide (often designated L-Cystine-di-β Naphthylamide) S-Benzyl L-Cysteinyl-β Naphthylamide S-Benzyl β Mercaptopropionyl β-Naphthylamide

Of these substances L-cystyl-di-β-naphthylamide was first synthesised and proposed as a model substrate for studies of the degradation of the amino-terminal end of oxytocin by Tuppy & Nesvadba (12) It has the same molecular configuration as the amino-terminal end of the oxytocin molecule. It should be attacked by a thiol-oxido-reductase activity and by an amino-peptidase activity The attack by a thiol-oxido-reductase cannot be detected by any simple method but the attack by an amino-peptidase is easily detected, because it results in liberation of β-naphthylamine and free β-naphthylamine can be detected and quantitated by the Bratton-Marshall method (1939)

In the studies with L-cystyl-di-β-naphthylamide the principle of enzyme inhibition was utilized. Thus the degradation of L-cystyl-di-β-naphthylamide should be inhibited upon addition of oxytocin. It should also be inhibited on addition of deamino-oxytocin Deamino-oxytocin, due to its lack of an amino-group cannot be attacked by an amino-peptidase but it should be attacked by a thiol-oxido-reductase. This means a competition with the liberation of β-naphthylamine if one assumes that human myometrium acts in the same way as that reported for liver preparations and rat uterine preparations, mentioned in the introduction. Therefore one part of this work was aimed at studying if and how oxytocin and deamino-oxytocin inhibit the liberation of β-naphthylamine from L-cystyl-di-β-naphthylamide by uterine extracts.

The theoretical intermediate after attack of the oxytocin molecule by a thiol-oxido-reductase is the linear polypeptide L-cysteinyl-L Tyrosine For this intermediate S-benzyl L-cysteinyl-β-naphthylamide (15) would be a model substrate It cannot be attacked by a thiol-oxido-reductase but it can be attacked by an amino-peptidase with liberation of β-naphthylamine It has already been shown that it is attacked by amino-peptidases, present in human liver (15) Thus if incubation of this substrate with uterine extracts results in the liberation of β-naphthylamine it can be taken as evidence that there is present an amino-peptidase activity capable of attacking oxytocin after rupture of its disulphide bridge Moreover further evidence for this conclusion would be if incubation of S-benzyl-β mercaptopropionyl-β-naphthylamide with uterine extracts does not result in the liberation of β-naphthylamine The only difference between this latter and the former model substrate is the absence or presence of an amino-group Therefore another part of this work involves incubations of S-benzyl-L-cysteinyl β-naphthylamide and S-benzyl-β-mercaptopropionyl-β naphthylamide with uterine extracts, to see, whether or not β naphthylamine is liberated.

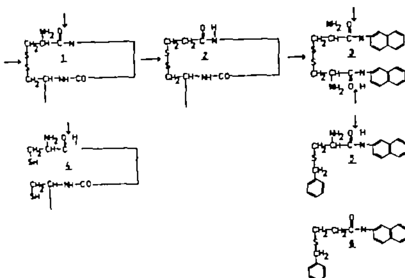


Fig. 2. Substrates used. 1 = Oxytocin; 2 = Desamino-oxytocin; 3 = L-Cystyl-di-β-Naphthylamide; 4 = Theoretical intermediate (Cysteinyl-Tyrosine) after rupture of the disulfide bond of oxytocin; 5 = S-Benzyl-L-Cysteinyl-β-Naphthylamide; 6 = S-Benzyl-β-Mercaptopropionyl-β-Naphthylamide. Arrows show points of enzymic attack.

The molecular formulae of the various substances used are shown in Fig. 2.

MATERIAL AND METHODS

The chemicals were of analytical reagent grade. The organic solvents were not further purified. The distilled water was glass-distilled.

1. The substrate

L-Cystyl-di-β-naphthylamide was obtained from Firma Serva, Vienna. S-benzyl-L-cysteinyl-β-naphthylamide and S-benzyl-β-Mercaptopropionyl-β-naphthylamide are not available commercially and had to be synthesized. The syntheses are described in the experimental section. A pure oxytocin preparation, 450 U/mg, and a less purified preparation of desamino-oxytocin, 350 U/mg, were used. These two latter preparations were obtained from Sandoz AG, Basel.

2. The myometrial extracts

Biopsies were taken from isthmus or corpus uteri of healthy pregnant women operated upon for such reasons as therapeutic abortion or contracted pelvis. The women were not in active labour when the biopsies were taken and no oxytocin were administered to them before sampling. The myometrial samples from non-pregnant women were taken at operations for fibroids. The biopsies were taken from apparently normal myometrium.

The samples were immediately rinsed with ice-cold 0.9% sodium chloride solution, dried on filter paper, weighed and within 10 minutes transferred to an ice-box at −20°C where they were kept until used for extraction. Storage time never exceeded one month.

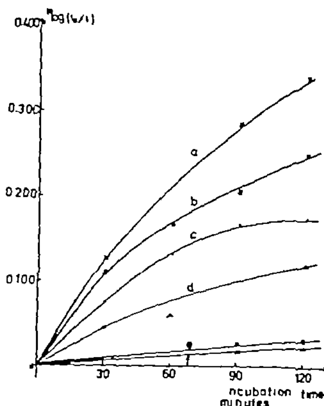
The extraction was performed in a cold room at +4°C, where all equipment and solutions were always stored. The weighed amount of the tissue was cut in pieces and ground in a mortar with sand and 0.9% sodium chloride solution for about 10 min. About 5 g of sand was used per g of tissue. 10 ml of 0.9% sodium chloride solution was used per g of tissue. This gives a concentration of 100 mg of tissue per ml of saline. The homogenate was then centrifuged for about 10 min at about 600 g. The supernatant was withdrawn and stored in the cold room until use. It was used for incubations within 2 hours.

3. The procedure for determination of enzyme activities

The methods described by Melander (7, pp. 39–55) and Rydén (9, pp. 27–31) were followed with some modifications. Only deviations from these methods will be mentioned here. For other details the reader is referred to these works.

A phosphate buffer pH 6.0, 0.067 moles/litre was used. The pH of the incubation mixtures was checked by a glass electrode at the end of the incubations and found to vary between 5.8–6.2. Thus the incubations were always performed at a pH about 6.

The synthetic substrates were dissolved in 0.01 M HCl. They were thereafter diluted with phosphate buffer to such a concentration that 0.50 ml added to the incubation mixture would give the desired final concentration of substrate. In the work with oxytocin and desamino-oxytocin a concentrated stock solution was diluted with the phosphate buffer and thereafter added as 0.50 ml portions to the desired concentration. The concentration of myometrial supernatant in the incubation mixture was always 33 %.



F g 3 Progress curves. Myometrial extract from a nonpregna t woman. *a b c* contained initially and calculated 450 *μ g* of L-cystyl-di-β-naphthylamide per ml; *d, e f* initially and calculated 150 *μg* per ml. To and *d* was not added oxytocin. *b* and *e* contained *1 3 U* of oxytocin per ml and *c* and *f* 3 U of oxytocin per ml.

A typical incubation mixture was the following:

Into each of test tubes were pipetted in the following order:

0.50 ml substrate solution.
0 50 ml phosphate buffer
0 50 ml supernatant.

The test tubes were placed in ter bath at +37°C and shaken frequently. At certain time intervals (usually 60 m and 120 min) 1 ml sample was taken from the incubation mixture and pipetted into test tube containing 1 ml of the trichloroacetic-acid solution.

Since repeat d contr ls did not sho any difference between using correct blank or distilled wate the majority of the incubations were performed with distilled water as zero time control or blank.

In the determinations f liberated β-naphthylamine the time for dev lopment of the colour was shortened to 1 hour. The samples were read at 565 m in a Beckman spectrophotometer model B.

4 The nzyme unit

International units (U) were sed as measure of enzyme activity 1 micromoles of substrate degraded per minute during the initial stages f the reaction F convenience the ultimate activity as calculated in milli-nits (mU) per ml incubation mi

There is reason to believe that the enzyme systems present in the extracts are unstable. This instability as thoroughly studied by Rydén (9) who used the same crude extracts as were used in this work and L-cystyl-di-β-naphthylamide as substrate. He worked out a mathe matical formula for expressing enzyme activity in inter national units. For principles and details of the calculations see Rydén (9 pp. 3–42) and Melander (7 pp 4 46). Here merely a description of the ultimate formula used will be given.

x and x = the absorbancy alue at 565 nm after 1 and hours incubation respectively
E_0 = initial enzyme concentration.
K_s = the zero order v locity constant for the degradation of the substrate
K = the elocity constant for the inactivation of the enzyme system.
$(e^{-K}) = 0.54(x\ \ x)/x$
$-K = {}^e\log[0.54(\ -x)\ x]/{}^{10}\log$
$E_0K = 0.54x\ K_s/(1-e^{\ K})$.
$mU = 1000\,E_0K_s/60$.

EXPERIMENTAL

A The Syntheses

1 S-benzyl-β-mercaptopropionyl-β-naphth lamide

S-benzyl-β-mercaptopropionic-acid, 0 628 g, and β-naphthylamine, 1 055 g, ere mixed and dissolved in 7 ml of tetrahydrofuran. N,N -dicyclohexylcarbodiimide, 1 110 g, dissolved in ml of tetrahydrofuran, was added and the reaction mixture was kept over-night in refrigerator at +4 C, mechanically stirred with magnetic stirrer. A thick suspension resulted. Glacial acetic acid, 0.5 ml, as added and the insoluble N,N -dicyclohexylurea filtered off. The filtrate was concentrated b rotary evaporation under running to about 40°C to a mobile yellow il This oil w s dissolved in 10 ml of ethyl acetate and the solution filtered to remov the remaining N,N -dicyclohex lurea. The resulting filtrate was successively ashed in separating funnel in the following order ith 1 M HCl, distilled w ter KHCO distilled water It as thereafter dried for 30 minutes th anhydrous Na_2SO_4 in excess ethyl acetate. The solution was filtered and the filtrate concentrated by rotary evaporation under arming t about 40°C to dryness. The residue was suspended in an ice-cold solution of methanol distilled w ter (70 30) Some material adhered t the glass all and could not be suspended. The suspended residue was dissolved in 5 ml of warm methanol (5 ml portions) and filtered. The filtr te as cooled ith ice ater and crystallization started It as kept for 5 min refrige tor t +4°C and thereafter ml of distilled ter as added. After about 10 min the solution as filtered A brow filtrate and colourless substance resulted The subst nce as dissolved in 30 ml of warm methanol (5 ml portions) and recrystallized by keeping in the refrigerator at +4°C for about 1 hour The solution w filtered and the substance dried in acuum over $CaCl_2$ over-night. The yield was rather low 130 mg. M lting point 108–109°C (capillary tube not corrected). A sample was

hydrolyzed at 110°C ith 6 M HCl. The Bratton-Marshall procedure revealed free β-naphthylamine. With prussian blue indication and sodium nitro-prussid indication the presence of N and S could be evidenced. Another sample as dried over P_2O_5 for elemental microanalysis. Calculated for C 74.7 H 6.0 N 4.4 S 10.0. Found C 74.9 H 6.0 N 4.2, S 9.4 (duplicate determinations).

S-benzyl-L-cysteinyl-β-naphthylamide

N-benzyloxycarbonyl-S-benzyl-L-cystein, 6.90 g, and β-naphthylamine, ..86 g, were mixed and dessolved in 40 ml of tetrahydrofuran. N,N'-dicyclohexylcarbodiimide, 4.12 g dissolved in 20 ml of tetrahydrofuran, was added slowly and under cooling in an ice-bath. It as therefore kept over-night at room temp. A thick suspension resulted. ml of glacial acetic acid as added and the insoluble N,N'-dicyclohexylurea filtered off. The filtrate as concentrated in rotary evaporator under arming to about 40°C to dryness. The residue was taken up ith 5 ml of glacial acetic acid, armed to about 40°C and carefully diluted with 20 ml of cold, distilled ater. After about hours at 4°C and room temp crystallization appeared. The solution was filtered and the substance dissolved in 5 ml of glacial acetic acid under arming to about 40°C and recrystallized from 20 ml of cold, distilled ater. The substance as filtered off and dried over P_2O_5. The yield as 0.30 g and the melting point 163–16 °C.

Of the N-benzyloxycarbonyl-S-benzyl-L-cysteinyl-β-naphthylamide obtained by the foregoing procedure, 2.36 g as dissolved in 1 ml of 18% HB in glacial acetic acid. After standing for about 1 hour as concentrated by otary evaporation and the residue dissolved in 100 ml of ether. The product precipitated. After decantation the residue as dried in acuum and dissolved in arm, distilled ater. After addition of aqueous ammonia to final concent of 1% dusty suspension resulted. After cooling and stirring hite precipitate appeared hich as recrystallized f om ater. The yield as 0.70 g ith melting point of 9 94°C. Calculated for N 8.8. Found N 6.6.

B Enzyme Kinetics

I Progress curves

The time course of the enzymic reaction as studied ith arying concentrations of L-cystyl-di-β-naphthylamide ith and ith varying concentrations of oxytocin and deamino-oxytocin and ith xtracts from non-pregnant and pregnant omen from different stages of pregnancy. The progress curves had largely the same course but in some instances quite unexpected shapes of the curves occurred probably due to the low solubility of L-cystyl-di naphthylamide). Such experiments ere then repeated several times ith meticulous attention to the experimental conditions. Consistent result ere then obtained.

Some representative progress curves are show in Fig. 3.

Influence of substrate concentration

The effect on enzyme activity of different concentrations of L-cystyl-di-β-naphthylamide is show in Fig. 4.

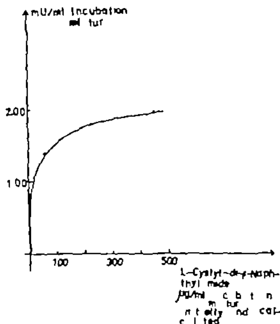


Fig. 4. Influence of substrate concentration on enzyme activity.

C The Solubility of L-cystyl-di-β-naphthylamide

It is ell-known fact that L-cystyl-di-β-naphthylamide has poor solubility in such media as phosphate buffer of pH 6. Different attempts have been made to solv this problem. Thus Rydén (9) continuously shook the test tubes in shaking apparatus during the incubations. Therefore the test tubes ere frequently shaken during the incubations in this study. Control experiments revealed that this manoeuvre gave more consistent results but even if the tubes were continuously shaken ith an apparatus could not prevent some precipitation of the substance.

Another approach as tried by Tuppy & Wintersberger (13) ho used ethyleneglycolmonomethylether (Methylcellosolve) to increase the solubility. This organic solvent as also tried in this study but with bad results. It seemed to inhibit the enzyme activity completely.

Human serum albumin was also tried to see if could act as protecting colloid and increase the solubility. However the preparation of albumin available contained something hich gave colour with the Bratton-Marshall procedure and therefore the problem could not be solved in this ay.

D Incubation i N_2-atmosphere and in Air

Parallel experiments ith L-cystyl-di-β-naphthylamide ere made in hich one set of test tubes ere continuously blow through ith stream of nitrogen before and during the incubation hereas the other set was lef open in contact ith air. No difference in the ate of degradation could be evidenced.

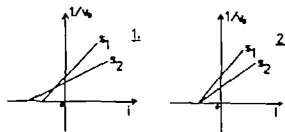


Fig 5 Plot according to Dixon (4) V_0 initial reaction velocity *i*, inhibitor concentration *S* and S_2 different initial substrate concentrations, *1* competitive inhibition *2* non-competitive inhibition.

RESULTS

I Oxytocin and deamino-oxytocin as inhibitors

The common way of studying the effect of in hibitors on enzyme activity is to plot the results with a range of substrate concentrations with and without a constant concentration of the inhibitor by the well-known method of Lineweaver Burk. This method, however requires knowledge of the initial substrate concentration. Due to the poor solubility of L-cystyl-di-β-naphthylamide exact knowledge of the substrate concentration cannot be obtained with this substance as substrate Therefore the method of Dixon (4) was used. In this method the initial reaction velocity (V_0 = mU) is determined with a series of inhibitor con centrations (I) keeping the initial substrate con centration constant. The method does not require exact knowledge of the substrate concentration—only that one is working at two different initial substrate concentrations The two theoretical graphs to be obtained are shown in Fig 5

The method of Dixon (4) was used with oxytocin and deamino-oxytocin as presumptive inhibitors. Supernatant from myometria of non-pregnant women and pregnant women in the first, second and third trimester of pregnancy were utilized. Typical results are shown in Fig. 6. It appears that oxytocin seems to act as a competitive inhibitor whereas deamino-oxytocin seems to act as a non-competitive inhibitor

II Incubations with S-benzyl L-cysteinyl-β-naphthyl-β-amine and S-benzyl-β-mercapto-propionyl β-naphthylamide

Parallel experiments were made in which super natants from myometria of non-pregnant women and pregnant women in the three trimesters were divided into two parts. Each part was incubated with the two substances respectively at a concentration of 650 μg/ml of the final incubation mixture at 37 C for 4 hours. In every case the Bratton Marshall procedure (3) could detect free β-naphthylamine after incubation with S-benzyl-L-cysteinyl-β-naphthylamide but could not evidence free β-naphthylamine after incubation with S-benzyl-β-mercaptopropionyl β-naphthylamide.

DISCUSSION

It is clear that this work must be regarded as a pilot study and that too much emphasis cannot be laid on the results obtained. However the results indicate that human myometrial extracts contain a thiol-oxido-reductase activity and an amino-peptidase activity The fact that deamino-

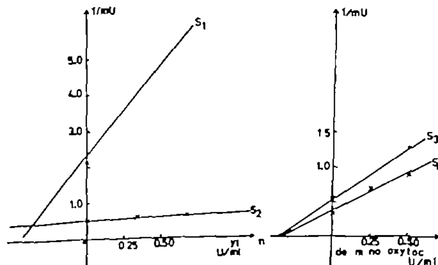


Fig 6 Inhibition of enzymic activity with oxytocin and deamino-oxytocin. Concentrations of L-cystyl-di-β-naphthylamide per ml incubation mixture initially and calculated were S_1 = 150 μg; S_2 = 450 μg, S_3 = 60 μg; S_4 = 40 μg.

oxytocin competed with the liberation of β-naphthylamine from L-cystyl-di-β-naphthylamide supports the conclusion that there is present a thiol-oxido-reductase activity. The fact that β-naphthylamine was liberated from S-benzyl-L-cysteinyl-β-naphthylamide but not from S-benzyl-β-mercaptopropionyl-β-naphthylamide supports the conclusion that there is present an amino-peptidase activity. It is also in accordance with the working hypothesis that oxytocin acted as a competitive inhibitor of the degradation of L-cystyl-di-β-naphthylamide.

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One reason why this work deliberately was kept at a stage of an orientating study is two serious drawbacks with the use of L-cystyl-di-β-naphthyl-amide and S-benzyl-L-cysteinyl-β-naphthylamide as model substrates. One is the poor solubility. The other is that the product formed, β-naphthyl-amine, is carcinogenic. It is therefore intended to repeat the study with other substrates. A better model substrate would be L-cystyl-di-L-tyrosin-amide synthesized and studied by Wintersberger et al. (14). It has almost exactly the same molecular configuration as the amino-terminal end of oxytocin. It has good solubility, the products formed on degradation are according to present knowledge not carcinogenic and it has been shown to be a competitive inhibitor of the degradation of oxytocin and L-cystyl-di-β-naphthylamide by plasma of pregnant women (11). The next step planned is therefore studies with this substance.

ACKNOWLEDGEMENT

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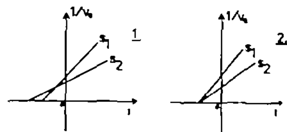


Fig 5 Plot according to Dixon (4) V_0 initial reaction velocity *I*, inhibitor concentration *S* and S_2 different initial substrate concentrations, *1* competitive inhibition *2* non-competitive inhibition.

RESULTS

I Oxytocin and deamino-oxytocin as inhibitors

The common way of studying the effect of inhibitors on enzyme activity is to plot the results with a range of substrate concentrations with and without a constant concentration of the inhibitor by the well-known method of Lineweaver Burk. This method, however requires knowledge of the initial substrate concentration Due to the poor solubility of L-cystyl-di-β-naphthylamide exact knowledge of the substrate concentration cannot be obtained with this substance as substrate. Therefore the method of Dixon (4) was used. In this method the initial reaction velocity (V_0 = mU) is determined with a series of inhibitor concentrations (I) keeping the initial substrate concentration constant. The method does not require exact knowledge of the substrate concentration—only that one is working at two different initial substrate concentrations. The two theoretical graphs to be obtained are shown in Fig. 5

The method of Dixon (4) was used with oxytocin and deamino-oxytocin as presumptive inhibitors. Supernatant from myometria of non-pregnant women and pregnant women in the first, second and third trimester of pregnancy were utilized. Typical results are shown in Fig. 6 It appears that oxytocin seems to act as a competitive inhibitor whereas deamino-oxytocin seems to act as a non-competitive inhibitor

II Incubations with S-benzyl L-cysteinyl β-naphthyl β-amine and S-benzyl-β-mercaptopropionyl β naphthylamide

Parallel experiments were made in which supernatants from myometria of non-pregnant women and pregnant women in the three trimesters were divided into two parts. Each part was incubated with the two substances respectively at a concentration of 650 μg/ml of the final incubation mixture at 37 C for 4 hours. In every case the Bratton Marshall procedure (3) could detect free β-naphthylamine after incubation with S-benzyl-L-cysteinyl-β-naphthylamide but could not evidence free β-naphthylamine after incubation with S-benzyl-β-mercaptopropionyl β-naphthylamide.

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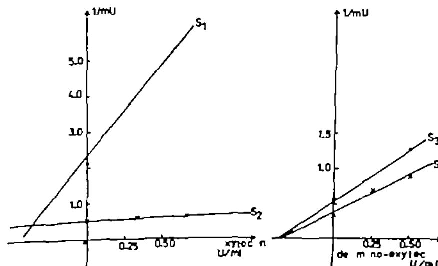


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CASE REPORTS

ENDODERMAL SINUS TUMOUR

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Gynaecological and Obstetrical Department (Head Jakob Lerørn), Viborg Hospital, Viborg, Denmark

Abstract A 32-year-old woman, who attended regularly for follow-up because of carcinoma in situ of the cervix uteri, developed symptoms of a rapidly progressing malignant process in the pelvis over a period of a few months. At operation large masses of tumour were found in both ovaries as well as diffuse metastases throughout the whole abdomen, and bilateral pleural effusions. Histological examination showed endodermal sinus tumour of characteristic structure. In spite of postoperative cytostatic therapy death occurred less than 9 months after the first symptoms. The theories about the origin of the tumour from germinal tissue are mentioned in the discussion. In 1939 Schiller (5) described a new type of malignant ovarian tumour for the first time. Because of its characteristic morphological structure, he presumed that it developed from mesonephric remnants. Later studies seem to show however that these tumours originate from extra-embryonic, endomesodermal tissue. Since there is doubt about the origin of these tumours, and since they are also uncommon, we feel justified in publishing the following case history.

CASE HISTORY

The patient was a 32-year-old married woman who had previously been well with the exception of an appendicectomy in 1957. The menarche was at the age of 13 and menstruation had been regular, lasting for 8 days every month. She had been pregnant twice and both pregnancies ended with a normal delivery, one in 1966 and one in 1969.

In January 1970, during hospital investigation for headache in the neurological department (where nothing abnormal was found) the patient complained that the last, normal menstrual period had been followed by continuous slight vaginal bleeding. A gynaecological examination showed that there was a superficial erosion of the cervix and retroflexion of the uterus. A fractional curettage was done and a cervical biopsy was taken. Histological examination showed endometrium in the proliferative stage as well as carcinoma *in situ* of the cervix. The following cone biopsy confirmed that there were no signs of invasive growth.

Approximately 1 month after the cone biopsy the patient came for her first visit as an outpatient and said that she was feeling well. The gynaecological examination revealed nothing abnormal and the cervix had healed completely.

At her next visit, approximately 2 months later, the patient complained of nausea and almost constant pains in both iliac fossae. She said that the pains had started after a normal menstrual period.

During this time she also had the impression that her abdomen had grown in size. Defaecation and micturition were normal and there was no feeling of tiredness and no loss of weight. On gynaecological examination it was impossible to define the uterus because of a large, partly cystic mass in front of it, and reaching to the level of the umbilicus. The impression was that there was ascites present. The routine laboratory tests were normal apart from an E.S.R. of 50 mm/h but an X-ray examination of the chest showed bilateral pleural effusions.

A laparotomy was done, approximately 3 months after the first operation, and more than 2 000 ml of bloody ascitic fluid was drained away. Everywhere in the abdomen there were large and small masses of tumour which grew around the inferior vena cava, and there were metastases in the liver. The uterus was normal, but the right ovary was enlarged to the size of a coconut, and the left ovary to the size of a mandarin, and both ovarian tumours were mobile. A bilateral oophorectomy was performed, but there was no attempt to remove all the tumour as the case was considered hopeless.

The histological report on the ovaries was as follows: Both ovaries have been transformed into very large masses of tumour. They are somewhat nodular and have a peculiar cheesy consistency when transected. The tumour masses from the left and the right side are histologically identical. The tissue consists of areas built up of fetal mesenchymal tissue with ample ground substance and stellate or fusiform cells. The findings re-



Fig 2

Macroscopically the tumour is often large and light with a lobulated surface There are alternating solid and cystic parts on the cut surface, which may give it a honeycomb-like, mucoid appearance

Endodermal sinus tumours in the ovaries constitute a rare, highly malignant, but well-defined entity in an otherwise extremely varied group of tumours arising from germinal tissue. Thus, Novak et al (3) *only found* 49 *in a review of* 1950 cases of different ovarian tumours, collected during a period of 20 years. The tumour occurs in young women, and in a series of 27 patients Neubecker (8) has found that the average age is 20 3 years *The disease progresses rapidly and* death most often occurs within 1 year after the first symptoms

The case published here does not vary from a typical case as both the clinical course and the histological study showed the characteristic traits of an endodermal sinus tumour as described by Teilum (6)

The histological picture is shown in Figs. 1 and 2

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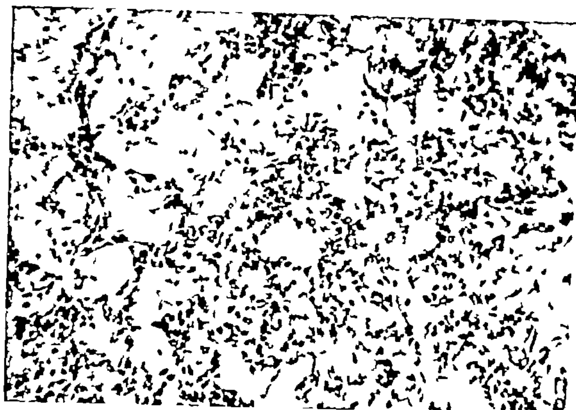


Fig 1

vealed large and small groups and anastomosing fibres of multicellular tissue, epithelial in appearance The cells arranged in solid masses, but in several places they surround imperfect slits or spaces. In a few places the cells are flattened. There is considerable nuclear poly morphism in the cells and they are on the whole hyper chromatic. In a few places very large nuclei are seen. Furthermore, several mitoses are observed, which are often atypical. Follicular structures cannot be demonstrated.

Histological diagnosis. Endodermal sinus tumour (Teilum) (signed. K. A. Lorentzen).

The postoperative course was uncomplicated and the patient was then transferred to the Radium Hospital in Århus, where she was treated with Endoxana® and Methotrexate® supplemented with Pyridoxine® and Prednisone® She managed quite well during the next 4 months, but after that there was growing tiredness, abdominal pain, nausea and loss of appetite Growth of the tumour masses was obvious, ascites and hydrothorax increased. Approximately 9 months after the laparotomy the patient was admitted again to the gynaecological ward in cachectic condition with large masses in the pelvis and in the right hypochondrium. There was dullness on percussion and reduced air entry over both lungs The patient grew rapidly worse until she died. Autopsy was not performed

DISCUSSION

As mentioned above Schiller (5) presumed that these tumours originated from the mesonephros He described the tumour histologically as a complex system of cystic spaces and communicating canals of various sizes, lined with endothelial cells that were low or cubic. In this meshwork there were a few glomeruli-like structures, made up of a central tissue bundle with a capillary surrounded by a cystic space lined with endothelial cells. The similarity between these structures and the fetal glomerulus led him to assume that the tumour arose from a mesonephric remnant.

Later after having made comparative studies of ovarian and testicular tumours Teilum (6 7 8 9) found that the tumour originated from germ cells and described it as an extra-embryonic yolk sac allantoic tumour showing stages in the phylogeny of the extraembryonic membranes He found the above mentioned complex system of communicating vascular canals comparable to the principal vascular mesoderm in the labyrinthine placenta, whereas the glomeruli-like structures were comparable to Duval's (1) so-called endodermal sinuses, i.e. diverticula of yolk sac endoderm which spread and separate around the branches of the allantoic vessels, where they enter the placental labyrinth in a rat or mouse Consequently Teilum considers this type of tumour in both the human ovary and testis as an extra-embryonic membranous tumour or endodermal sinus tumour

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TRIPLETS PREGNANCY WITH A NORMAL FOETUS AND A DICEPHALUS DIBRACHIUS SIRENOMELUS

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Abstract A two-headed monster and normal foetus in the same pregnancy have not been described previously in the literature. The delivery of case of this nature is reported and some speculations as to the aetiology of the formation of double monsters, based on experimental investigations of animal embryos, are outlined.

No earlier report of pregnancy with a double monster as well as a normal foetus has been found in the literature. The following description is of the delivery of such a case.

CASE HISTORY

A 23-year old primigravida was admitted in labour as an emergency to the gynaecological obstetrical department of Aalborg Hospital North, nine weeks before estimated term.

Thirty minutes after admission to the labour ward, the membranes ruptured spontaneously and clear liquor escaped, accompanied by prolapsed cord. Lower segment Caesarean section was immediately performed, and two-headed monster Dicephalus Dibrachius Sirenomelus weighing 990 g was delivered showing no signs of life. Following this, live male child presenting as breech (R S A) was delivered, weight 1900 g, length 47 cm, presenting no external malformations. The child died of the respiratory distress syndrome after thirteen days in respirator.

Two separate placentas were extracted; that of the monster measured 20 × 12 cm, weighed 200 g, and had cord length of 40 cm. The membranes were found to be thin and ragged. The placenta of the normal foetus measured 1 × 11 cm, weighed 400 g, with cord length of 34 cm. The membranes were complete. The puerperium was uneventful.

DISCUSSION

In the past decade delivery of a double monster has not been registered among the 12 000 deliveries that have taken place in this department. This is in agreement with the reports of the frequency of double monsters in hospital series being less than 1 50 000 (1 5 9). Two American analyses of birth certificates from large populations state the frequency of double monsters as 1 165 000 (11) and the frequency of liveborn double monsters as 1 200 000 (2).

The aetiology of the development of double monsters (4) has been studied by experimental investigation of animal embryos: hamster (6), zebrafish (7), and salmon (12) and retrospective investigation of pre-natal events (8). It is thought that the factors that trigger off the twin development by a fission process in a single egg, are changes in the micro-climate of the egg (in experimental animal investigations, for instance, low oxygen tension and temperature variations). If the egg is exposed to factors such as these in the third foetal week, this is the time when the risk of the fission process not reaching completion is the greatest, with a double monster ensuing. If the fission process proceeds without complications, a pair of monozygotic twins will be formed.

In the case reported here of presumably originally dizygotic twins, one might imagine that the implantation and growth of one egg has been optimal, while growth conditions, the micro-climate, for the other egg have been correspondingly impaired in the third foetal week, so that a fission process in progress has not reached completion.

It is known that double monsters, more often than monozygotic twins, exhibit dissimilarities, and this is well demonstrated in this case with the deformities on the one half. The occurrence of first branchial arch defects in double monsters

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AORTIC COARCTATION AND PREGNANCY

Leif Hillestad

From the Medical Department (Head, Prof. A. Hellem), Akershus Central Hospital, University of Oslo Oslo, Norway

Abstract. An 18-year-old primigravida was admitted in the seventh month of pregnancy with diagnosis of pre-eclampsia, based on recent rise of the blood pressure up to 175/110 mmHg. Oedema and proteinuria were absent. Examination revealed absent femoral pulses and no measurable blood pressure in the lower limbs. A harsh holosystolic murmur grade 4, was audible over the precordium. A collateral pulse was felt at the medial border of the left scapula. The chest film showed rib erosions and possible widening of the ascending aorta. A diagnosis was made of aortic coarctation with probable aortic aneurysm. To avoid the strain of labour Caesarean section was successfully undertaken. Subsequent aorto-angiography showed the presence of marked aortic coarctation, an aneurysm of the ascending aorta and markedly dilated innominate, left subclavian and mammary arteries. Surgical repair was undertaken eight weeks after delivery. The aortic stenosis had diameter of only 3 mm. Histologic examination of the removed specimen revealed marked atheromatosis, intimal fibrosis and mucoid degeneration. The findings thus justified the therapeutic procedure selected. The diagnosis of aortic coarctation reviewed and the management when found during pregnancy is discussed.

Aortic coarctation in pregnancy is rare, but should be borne in mind by every physician in charge of a pregnant woman. Unless recognized and appropriate treatment instituted the pregnancy can end in disaster. The main causes of complications and death are aortic rupture, bacterial endocarditis, congestive heart failure and rupture of intracranial aneurysms.

Diagnosis is easily made by the demonstration of arterial hypertension limited to the upper extremities. The present report is reminder of the basic elements of the diagnosis and of the therapeutic considerations to be undertaken when aortic coarctation is found in pregnancy.

CASE REPORT

An 18 year-old married primigravida was admitted to our Department of Gynecology and Obstetrics on October 8, 1970 with diagnosis of pre-eclampsia.

The last menstrual period had started on February 23 and the term estimated date of delivery as December 3 1970.

She had previously been healthy and the early months of pregnancy had been uneventful.

During the last eeks prior to the admission she had noted slight, but persistent frontal headache. At the same time the physician in charge had observed small rise of the blood pressure, which on tw occasions had reached distinctly abnormal level of 175/110 mmHg. No oedema or proteinuria could be found.

On admission the supine blood pressure was 180/110 mmHg. There are modest hypertensive eye ground changes, but normal urine and no oedema. A harsh systolic murmur as audible over the precordial area. The electrocardiogram was normal and the examinations other were unrevealing.

She was treated by rest in bed and salt restriction, and subjected to ordinary surveillance. The blood pressure remained at 160/110 mmHg during the first days, and antihypertensive treatment was therefore started. Hydralazine and reserpine and later chlorthalidone, were without convincing effect. The mean value of series of blood pressure measurements was 190/110–100 mmHg.

Due to the occurrence of bouts of tachycardia and the cardiac murmur observed earlier, the possibility of congenital heart disease as well as the hypertension was entertained. The cardiologist as therefore called upon. His examination showed blood pressure in both arms of 170/120 mmHg. A vigorous pulsation of the carotids was noted. The heart beat could not be felt. A holosystolic murmur with mid-systolic maximum was audible over the precordium. It had its peak intensity of grade 4 at the left sternal border in the 3rd intercostal space. The second aortic sound was accentuated, the second pulmonary sound was normal. No pulsation was present in the femoral arteries, and measurement on the thigh ith the appropriate cuff failed to demonstrate any blood pressure. Further examination revealed distinct collateral pulsations at the medial border of the left scapula, and the chest film showed the typical erosions of the lower borders of the ribs. Moreover the film disclosed suspect idening of the ascending aorta.

The presence of an aortic coarctation with possible aortic aneurysm made it clear that any circulatory strain was to be avoided as far as possible. A surgical repair of the coarctation seemed out of question, because the large fetus would probably make the operation as well



Fig 1



Fig 2

has been examined by Markovic (10) who found only one other published case besides his own.

In the case described here a correct diagnosis had not been established ante partum. It is evident from the literature that the diagnosis is often first made when labour is in progress or accidentally when an X ray examination is carried out because of a suspected twin pregnancy and even then the condition may be overlooked (9)

The mode of delivery chosen on account of the prolapsed cord was timely both as regards the presence of twins and the monster and is recommended by most authors for delivery of double monsters (1 5 9) Delivery has been reported through the lower (9) as well as through the upper uterine segment (1)

The prognosis for mature double monsters has improved greatly in recent years, successful separation of most types having been accomplished (3 9)

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pregnancies in 9 unoperated women with 21 pregnancies in 8 operated women are also convincing. In the first group 70% of the pregnancies were associated with cardiovascular complications and with a fetal death rate of 60%. Corresponding figures from the other group were 15 and 9% respectively.

Thus strong evidence exists for operating on young women with aortic coarctation prior to pregnancy.

If the diagnosis is first made during pregnancy treatment must vary according to the circumstances. Until the twentieth week reconstructive surgery has a good chance of success (7). Later on the size of the fetus will be a relative obstacle for an uneventful recovery period due to, among other factors, the effect upon the respiratory movements. Usually the circulatory stress undergoes a significant decline in the last weeks before delivery. If the blood pressure is only moderately elevated and no dangerous complications are anticipated nothing ought to be done. In most instances, however the presence of weakened aortic wall, intracranial aneurysms or latent heart failure is impossible to exclude. A Caesarean section is then much to be preferred to avoid the strain of labour. It is necessary to stress that therapeutic abortion and sterilization both are outdated as treatment. They belong to the preoperative era.

At any stage of the pregnancy treatment has to be carried out with due respect to the physiological alterations, which take place. From the third to the seventh month of pregnancy circulation is successively accelerated, and blood volume and cardiac output increase by 30 to 50%. Although mean blood pressure remains fairly constant, this represents the first critical period for the patient with aortic coarctation. The second critical period is labour itself, because blood pressure and cardiac work increase by about 20% at the peak of each uterine contraction.

According to the literature aortic rupture is most frequent in the third trimester and in multiparae, while labour itself seems less dangerous (6). However deaths from this complication have been observed during labour or just after delivery (1 6). The strain of labour is therefore not to be underestimated in such patients.

The major threat to the pregnant woman with an unoperated aortic coarctation is the aortic rupture. This obviously occurs as a result of the high aortic blood pressure and the concomitant degenerative changes of the aortic wall. In many respects these changes are similar to those described in cystic medionecrosis. The pathogenesis of these changes in aortic coarctation is unknown. They may be pressure-mediated or represent another congenital abnormality. The predominance of this complication is illustrated by the fact that of all aortic ruptures under the age of forty years more than 30% are associated with coarctation (6).

This congenital anomaly is often associated with other congenital defects, the most frequent of which are bicuspid aortic valve, persistent ductus arteriosus and ventricular septal defect. Especially the presence of a bicuspid aortic valve must be excluded or if present, followed up because it is often the cause of severe aortic stenosis in adult age. The other defects are to be corrected surgically together with the coarctation.

The present report provides the diagnostic clues of aortic coarctation and the considerations to be made when this condition is found in pregnancy. Evidence is also presented that antihypertensive therapy is ineffective in this condition.

A great responsibility is laid upon the physician in charge of the pregnant woman. Strict rules for normotension are to be held since borderline hypertension is often encountered both in coarctation and pheochromocytoma (2). In every case of hypertension it is essential to make a thorough search for underlying mechanisms.

Aortic coarctation can initiate dangerous complications in pregnancy if not detected. It is a curable condition. Its rare occurrence makes it a medical diagnostic challenge.

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Fig 1 Angiocardiography demonstrates the aneurysm of the ascending aorta and the coarctation of aorta situated just below the rigin of the left subclavian artery

as the postoperative period hazardous. Although the worst phase of pregnancy with regard to circulatory stress was over howeve there was reaso to fear labour with its intermittent blood pressure rises. It was therefore de cided t do a Caesarean section, which on October 30



Fig The dilated innominate left subclavian and mammary arteries are clearly visible. The right mammary ar tery is seen to anastomose with the epigastric artery Again the site of the coarctation can be observed.

resulted in a normal baby boy who initially suffered from cyanosis, but ultimately recovered completely

A fortnight later the mother was subjected to an aorto-angiography This demonstrated the presence of a conspicuous dilatation of the ascending aorta and a tricuspid aortic ostium (Fig. 1) A marked dilatation was also seen of the innominate, left subclavian and both mammary arteries (Fig.). The latter formed distinct anastomoses with the pigastric branch of the external iliac arteries. On all pictures the coarctation was clearly visible on below the origin of the left subclavian artery The stenosis was narrow but not complete as a small amount of contrast medium could be seen to pass through it.

The patient was discharged and readmitted two months later f reconstructive surgery During the operation the coarctation was removed and an end-to-end anastomosis performed. The stenosis had a diameter of only 3 mm. Histologic examination of the removed specimen showed the presence of marked theromatosis, intimal fibrosis and mucoid degeneration of the aortic wall.

The patient made an uneventful recovery with subsequent blood pressure of 130/80 mmHg in the arms and of 130/100 mmHg in the legs. She is under continued control in order to follow the course of the aneurysm of the ascending aorta. No advice has so f r been given with regard to future pregnancies.

COMMENTS

The true incidence of aortic coarctation in pregnancy is unknown, but obviously is of a very low order It has been found twice among 9 000 pregnant women (3) and of 168 patients under going cardiac surgery in pregnancy only five suf fered from aortic coarctation (4).

None the less the disorder is important to recognize, because dangerous complications of the pregnancy thereby can be minimized. This is illustrated by the study of 228 patients (1 5 8), who went through the pregnancy without surgical repair of their coarctation. Serious complications were noted in 43 patients and 15 died The maternal deaths were caused by aortic rupture in 9 cerebrovascular accident in 2, bacterial endocarditis in _ and congestive heart failure in _

In contrast the death rate among 60 patients who had been operated for their coarctation prior to the pregnancy was only 1 7 (8).

Until now 10 patients have undergone surgical repair during pregnancy (8) In nine the pregnancy remained free of complications. The one who died was operated on during the first month and succumbed in the seventh month from an aneurysm of the aorta at the anastomotic site. The results of a study (5) comparing the course of 32

pregnancies in 9 unoperated women with 21 pregnancies in 8 operated women are also convincing. In the first group 70% of the pregnancies were associated with cardiovascular complications and with a fetal death rate of 60%. Corresponding figures from the other group were 15 and 9% respectively.

Thus strong evidence exists for operating on young women with aortic coarctation prior to pregnancy.

If the diagnosis is first made during pregnancy treatment must vary according to the circumstances. Until the twentieth week reconstructive surgery has good chance of success (7). Later on the size of the fetus will be a relati e obstacle for an uneventful recovery period due to, among other factors, the effect upon the respiratory movements. Usually the circulatory stress under goes significant decline in the last weeks before delivery. If the blood pressure is only moderately elevated and no dangerous complications are anticipated, nothing ought to be done. I most instances, however, the presence f a weakened aortic wall, intracranial aneurysms or latent heart failure is impossible to exclude. A Caesarean section is then much to be preferred to od the strain of labour. It is necessary to stress that therapeutic abortion and sterilization both are outdated as treatment. They belong to the preoperative era.

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Announcement

Second Danube Congress for Gynecology and Obstetrics, 6–8 June 1972, Budapest, Hungary

Main Topics: Pregnancy and extragenital (accidental) diseases; Gynecological endocrine diseases; Free communications.

Official languages: Hungarian, Russian, English, German.

Deadline: 15 February 1972, submission of summaries; 31 March 1972, submission of papers.

Information. Second Danube Congress, Secretary Mrs Agnes Rubányi, Office for Conference Organization (MOTESZ) Budapest V Kossuth Lajos tér 4 Hungary

The 2nd World Congress on Ultrasonics in Medicine will take place in *Rotterdam on 4–8 June 1973* under the auspices of the Rotterdam Medical Faculty and the World Federation for Ultrasound in Medicine and Biology

Themes: Current developments of ultrasonics in connection with the following fields: Cardiology Gynaecology Internal Medicine Neurology Ophthalmology and Physics.

Papers can be read in English, French and German during Invited Speakers sessions. At other times preferably in English. The deadline for Abstracts is January 1973.

For information write to The Secretariat of the 2nd World Congress on Ultrasonics in Medicine c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, Netherlands.

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TREATMENT OF CANCER OF THE VULVA

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Abstract. The results of various forms of primary treatment of cancer of the vulva during the decade 1958–1968 in the Radium Centre, Århus Municipal Hospital, University of Århus, are submitted. The series consists of 67 patients classified by the T-N-M system (U.I.C.C. 1967). Comparison of 29 patients treated by vulvectomy and node dissection with 26 patients treated by less extensive surgery without node dissection showed 19% higher corrected 4 year survival and 14% lower nodal recurrence rate in the former group although according to the T-N-M classification it must be presumed to have contained rather more advanced cancers. There was quite marked difference between the clinical assessment of the regional lymph nodes and the histological findings at dissection. This applied to the histologically positive as well as negative lymph nodes. A review of the literature shows that the present results are in keeping with previous publications which have shown that the treatment of choice in cancer of the vulva is still total vulvectomy with routine dissection of the superficial and deep inguinal lymph nodes, supplemented by dissection of the iliac lymph nodes, if histological examination during the operation shows positive lymph nodes in the inguinal region.

Prickle-cell/squamous-cell carcinoma of the vulva (called cancer of the vulva in this paper) makes up about 90% of all malignant tumours arising in the vulvar region (1, 6). Traditionally cancer of the vulva is considered to have a poor prognosis, the recurrence rate being high and node involvement early (4, 5, 15). Therefore, treatment has to be extensive and radical, if there is to be any hope of a cure. However insistence on this demand comes up against the difficulty that most of the patients are elderly and often have other diseases, particularly of the cardiovascular system, which increase the therapeutic risk.

These factors are reflected in the marked variation found in the primary treatment of vulvar carcinoma. However since the first publications of the excellent results achieved by radical vulvectomy plus dissection of the regional lymph nodes (2, 10, 14) this way of treatment seems to have been used more often than any other treatment. Several authors in recent years have published the results of this treatment (1, 3, 9). Various surgical techniques have been described in some detail by different authors, (7, 11, 12).

The purpose of this paper is to submit the results of different forms of treatment in a series of vulvar cancer classified by the T-N-M system adopted by the U.I.C.C. in 1967 and analysed statistically by Nohrman's formula for annually corrected survival.

MATERIAL

During the period 1.1.1958 to 1.1.1968 a total of 69 women were admitted to the Radium Centre, Århus Municipal Hospital, University of Århus, for primary treatment of cancer of the vulva. Two received no treatment because of their poor general condition. One died of uraemia two weeks after the diagnosis. The other one had heart disease with severe signs of decompensation at the time of diagnosis and succumbed to her cardiac disease 2 months later. Both tumours were large (T_3 and T_{3M}) with fixed regional lymph nodes (N_3).

During the same period 13 patients were admitted for treatment of recurrent cancer of the vulva after primary treatment elsewhere and another 13 with vulvar malignancy of another histological type. Eight had basal-cell carcinoma, four malignant melanoma, and one patient had a malignant haemangiopericytoma.

Fig. 1 gives the age distribution of the 67 patients treated primarily for cancer of the vulva in the Radium centre. The average age was 63.5 years, range 20–86 years.

SYMPTOMS AND SIGNS

The most common symptoms were itching, burning, or pain in the vulvar region, or else the pa-

lymph nodes unilaterally or bilaterally but clinically it could not be definitely decided whether there was a suspicion of nodal involvement (N1+N2). In 10 patients there was a suspicion or clinical evidence of regional node involvement (groups N1*b*+N2*b*+N3). Two patients could not be classified by the T-N system owing to deficient data in the case records.

By the M classification the patients were distributed as follows: M0 66 patients; M1 1 patient.

TREATMENT

Since 1962 the standard treatment has been total vulvectomy and bilateral dissection of the superficial and deep inguinal as well as external iliac lymph nodes. The operation is carried out in one stage, removing the regional lymph nodes through separate incisions. In most cases the common iliac lymph nodes are removed as well. No departures from this treatment were made except in cases of inoperable tumours, which were treated by radiotherapy and in patients who were not considered fit for extensive surgery because of advanced age or severe concomitant disease. In these cases surgery was restricted to vulvectomy or a minor procedure (hemivulvectomy excision of the tumour).

From 1958 to 1962 the treatment was somewhat more varied. A number of patients had electrocoagulation of the primary tumour and compared with the period after 1962 a relatively larger number had minor surgical procedures.

In cases treated primarily by radiotherapy this treatment was in the form of roentgen irradiation of the primary tumour (165–200 kV), total doses ranging from 2 100 to 4 000 R (measured in air) in about 3 weeks. This treatment was also given to the inguinal lymph nodes in two cases. Roentgen irradiation of the primary tumour was supplemented in two cases with contact radium therapy.

During the entire period 1958 to 1968 the patients were distributed as follows by form of treatment:

(A) Total vulvectomy and dissection of regional lymph nodes, 29 patients,
(B) Excision, electrocoagulation, hemivulvectomy or vulvectomy without dissection of the regional lymph nodes, 26 patients;
(C) Electrocoagulation + dissection of the regional lymph nodes, 3 patients;
(D) Excision + roentgen irradiation of primary tumour 1 patient,
(E) Roentgen irradiation of primary tumour (in 2 cases supplemented by roentgen irradiation of the regional lymph nodes) 8 patients.

Group (A), comprising 29 patients, had the following treatment of the regional lymph nodes:

Bilateral dissection of the inguinal and iliac lymph nodes, 22 patients;
Bilateral dissection of inguinal nodes only 4 patients;
Unilateral dissection of inguinal and iliac nodes, 1 patient,
Unilateral dissection of inguinal nodes only 2 patients.

Two of the above mentioned patients had postoperative roentgen irradiation of the regional lymph nodes, but this cannot be attributed any importance as both patients had had radical surgery. The motivation for omitting iliac dissection in 6 patients was that at the operation the inguinal nodes were found to be normal (not, however, examined histologically during the operation). All 3 patients treated by unilateral nodal dissection were from the early part of the period, and bilateral dissection was omitted because the nodes on the untreated side had been assessed as clinically normal.

The patients of the various treatment groups were distributed in the T-N system as shown in Table II. (In this table number of T-N combinations are collected in groups estimated to be fairly uniform as regards prognosis.) It will be seen that among 29 patients treated by vulvectomy and node dissection (group A) 4 were clinically suspected of regional node involvement (N1*b*–N2*b*–N3), while among 26 patients treated by less extensive surgery (group B) only one had clinically suspicious regional lymph nodes.

Table III sets out the relationship between the clinical and operative findings in respect of the regional lymph nodes. Among the 29 patients treated by vulvectomy and dissection of the regional nodes there were 15 in whom no palpable inguinal nodes were found and 2 in whom the nodes were palpable but not suspicious. Among these 17 patients 3 exhibited involvement of the

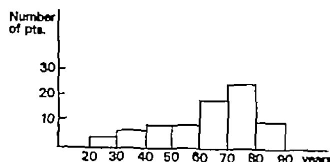


Fig 1 Age distribution of 67 patients with cancer of the vulva.

tients noticed an ulcer or nodule bleeding, discharge, or burning on micturition. As a general rule the patient noticed the tumour because of these associated symptoms or else they did not consult a doctor until such symptoms occurred. Thus, there were only 6 tumours without associated symptoms. In 23 cases there was kraurosis and/or leucoplakia (35%) In 12 out of these 23 patients the symptoms had existed for many years.

Duration of Symptoms at Time of Diagnosis

In 58 of the 67 patients the duration of symptoms at the time of diagnosis could be established with fair certainty Symptoms for a maximum of 3 months, 23 patients (about 40%) symptoms for a maximum of 1 year 24 patients (about 42%) and symptoms for more than 1 year 11 patients (about 19%)

Classification

The classification was by the T N M system adopted by U I C.C. in 1967 and given below in full

T Primary tumour

TiS Pre-invasive carcinoma, so-called carcinoma *in situ*.

T0 No evidence of primary tumour

T1 Single tumour not more than 2 cm in its largest dimension.

T2, Single tumour more than 2 cm, but not more than 5 cm in its largest dimension.

T3 Single tumour more than 5 cm in its largest dimension *or* tumour of any size with extension to vagina not more than 2 cm in length, *or* to anal canal without involvement of mucosa, *or* with extension to urethra.

T3(m) Multiple tumours covering an area of not more than 10 cm in diameter

T4 Single tumour of any size with extension to vagina more than 2 cm in length, *or* to anal canal with involvement of the mucosa, *or* to recto-vaginal septum *or* to other neighbouring structures.

T4(m) Multiple tumours covering an area more than 10 cm in diameter

N Regional lymph nodes

The clinician may record whether palpable nodes are considered to contain growth or not.

N0 No palpable nodes.

N1 Movable homolateral nodes.

N1a Nodes not considered to contain growth.

N1*b* Nodes considered to contain growth.

N2, Movable contralateral or bilateral nodes.

N2a Nodes not considered to contain growth.

N2*b* Nodes considered to contain growth.

N3 Fixed nodes.

M Distant metastases

M0 No evidence of distant metastases.

M1 Distant metastases present.

The 67 patients with cancer of the vulva were distributed by the named T-N classification as shown in Table I. As is apparent from the table there were at the time of diagnosis 41 patients in group N0 i.e. without palpable inguinal lymph nodes. Five patients had palpable but not suspicious regional lymph nodes (groups N1*a* and N2*a*) Nine patients had palpable but movable

Table I. *Distribution by the T–N system of 67 patients with cancer of the vulva immediately before primary treatment*

	T_{is}	T	T	T	T	T_{3m}	T	T_{4m}	X^b	
N_0	1	[illegible]	14	16	6	1	1			41
N			2	1	2		1			6
N_{1a}										0
N_{1b}			1	1	1					3
N				1	2					3
N_{2a}			3	1	1					5
N_{2b}							2			2
N_3				1	1		1			3
X^b							1		1	2
	1		20	21	13	1	8	0	1	

Primary tumour completely removed by biopsy
b U known.

Table IV *Results of vulvectomy and node dissection, less extensive surgery without dissection, and radiotherapy in terms of 4 year survival (crude survival)*

Group A		
Vulvectomy + nodal dissection	Total 29 pats. with minimum follow-up period of 4 years	66 %
Group B		
Less extensive surgery without dissection	Total 23 pats. with minimum follow-up period of 4 years	39 %
Group C		
Radiotherapy	Total 8 pats.	0 %

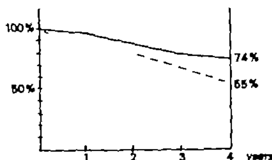


Fig. 2. Annual corrected survival rate, calculated according to Nohrman formula in the surgically treated groups. (A) — and (B)

cancer while one is alive without recurrence. The only patient who was treated by excision of the primary tumour supplemented by irradiation of the tumour site has died of cancer

Furthermore, the therapeutic results of vulvectomy and node dissection (group A) and of less extensive surgery without node dissection (group B) were compared in Fig. 2. In both groups the annual corrected survival was calculated by Nohrman's formula (survival rate for the nth year) $100 \quad (1-Q_{n-1}) \times (1-Q_n)$ where $Q = C/(C+L+I/2)$, C = number of deaths from cancer in the nth year after the first day of treatment, I = number of deaths from intercurrent diseases in the nth year after the first day of treatment, and L = number of survivors at the end of the nth year after the first day of treatment.

Recurrences

Table V lists the number of cases with recurrent cancer in the groups treated by vulvectomy and node dissection (group A) and by less extensive surgery without node dissection (group B). The cases with recurrences are divided into 3 groups on the basis of the site of the recurrence: local only, in regional lymph nodes only and local as well as in regional lymph nodes. It is evident from the table that among 29 patients treated by vulvectomy and node dissection 7 developed metastases in the regional lymph nodes (24 %), while among 26 patients treated by less extensive surgery 10 developed metastases of the regional lymph nodes (38 %).

The 3 patients with local recurrences only were treated by excision, and all are alive without recurrence. The 17 patients with nodal metastases received different forms of treatment: 5 were treated by roentgen or cobalt irradiation, 4 by dissection, 1 by electrocoagulation, while 6 received no treatment (5 had distant metastases at the time when the nodal recurrence was detected). All 17 died with cancer

Table VI gives a more detailed analysis of the group treated by vulvectomy and node dissection. It is divided into 3 sub-groups by histological findings in the regional lymph nodes. The follow-up period is a minimum of *4 years* on the 29 patients. 19 patients had no metastases, and of these 4 died with cancer within 4 years. 10 had involvement of the inguinal + iliac nodes or of the inguinal nodes only: out of this group 3 died with cancer within 4 years. The number of patients is too small for a more detailed evaluation of a difference, if any, due to the presence of iliac node involvement.

Postoperative mortality

In the group of 29 patients treated by vulvectomy and node dissection there were no postoperative

Table V *Recurrence rate among the surgically treated patients*

	Local only	Regional lymph nodes only	Local + regional lymph-node metastases
Group A			
Vulvectomy + dissection	3	5	2
Group B			
Less extensive surgery without dissection	0	7	3

Table II. *Relation between primary treatment and T–N category of 67 patients*

	No palpable lymph nodes			Nodes palpable but not suspicious		Palpable nodes, no decision concerning suspicion		Suspicious nodes			
	T_{is}–To No	T (1–2) No	T (3–4) No	T (1–2) N (1*a*–2*a*)	T (3–4) (N 1*a*–2*a*)	T (1–2) N (1–2)	T (3–4) N (1–2)	T (1–2) N (1*b*–2*b*–3)	T (3–4) N (1*b*–2*b*–3)	Not stated	Total
Group A Vulvectomy + dissection	2	10	3	2		3	4	3	1	1	29
Group B Less extensive surgery* without dissection	1	16	4	2	1		1		1		26
Group C Electro-coagulation + dissection		2				1					3
Group D Excision + local Radiotherapy		1									1
Group E Radiotherapy		1	1						5	1	8
Total	3	30	8	4	1	4	5	3	7	2	67

* Less extensive surgery comprises all patients treated by excision, hemivulvectomy vulvectomy and electrocoagulation without dissection of the regional lymph nodes.

inguinal nodes at operation. Among 7 patients with palpable regional lymph nodes in whom no clinical assessment as regards suspicion was made (N1–N2) 4 were found to have node involvement at operation. Among 4 patients with clinically suspicious regional lymph nodes (N1*b*–N2*b*–N3) 2 showed histologically demonstrable involvement. In one case the clinical assessment could not be established because of deficient data in the case records. This patient was found to have node involvement at operation. In all 10 patients with histologically demonstrated regional node involvement, the secondaries were found in the superficial inguinal nodes—in 6 of them also in the deep inguinal nodes and in 3 also in the iliac nodes.

Table III *Relation between clinical N classification and the operative findings of regional lymph-node metastases in 29 patients treated by vulvectomy and dissection*

Clinical N category	No. of pats	No. of pats. with histologically demonstrable lymph-node metastases at operation
N–O	15	2
N (1*a*–2)		1
N (1 2)	7	4
N (1*b*–2*b*) N 3	4	2
X	1	1

THERAPEUTIC RESULTS

In the 67 cases there is a minimum follow-up period of 4 years.

Survival

The results are listed in Table IV stated as the crude survival rate for the 3 therapeutic methods: Group A vulvectomy and node dissection (66) group B less extensive surgery without dissection (39%) and group C, radiotherapy (0%) It may be added that out of the 3 patients treated by electrocoagulation of the primary tumour plus dissection of the regional lymph nodes _ died of

is slightly greater in group A than in group B 17% and 11% respectively whereas the rate of regional lymph node recurrences is greater in group B than in group A (38% and 24%).

Although this difference of 14% is not statistically significant owing to the small numbers of patients in the two groups, one fact is shown clearly by these percentages: the greater rate of regional lymph node recurrences in group B is not due to a higher rate of local recurrences in this group.

Table VI shows the fate of the 10 patients who had histologically demonstrable metastases in the regional lymph nodes at the time of primary treatment. Three of these patients died with cancer within the observation period of 4 years.

This mortality of 30% is in sharp contrast to the mortality of 100% of the 17 patients who developed regionary lymph node metastases after the end of the primary treatment.

It should be noted that all patients were seen at regular intervals after the primary treatment, and treatment of recurrences instituted as soon as these were diagnosed. Therefore there is no obvious explanation of the fact that the prognosis is so much poorer when regional node metastases occur as recurrences than when they are present at the time of primary treatment.

Although the number of patients is too small to allow any definite conclusions, the lower rate of nodal recurrences in the group treated with vulvectomy plus dissection (group A, Table IV) indicates the value of this treatment, especially when the very poor prognosis of the nodal recurrences is born in mind.

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Table VI. *Four year survival among 10 patients with and 19 patients without histologically demonstrable regional lymph-node metastases. Treatment in all 29 cases vulvectomy + node dissection*

Histologically demonstrated metastases in the regional lymph nodes	Died of cancer	Died of inter current disease	Survived for at least 4 years	Total no. of pats.
Inguinal + iliac	1	1	1	3
Inguinal only	2		5	7
None	4	2	13	19
Total	7	3	19	29

deaths. In the group of 26 patients treated by less extensive surgery there was one postoperative death. Thus, the total postoperative mortality among the surgically treated patients was 1.9%

Healing

Among the 29 patients treated by vulvectomy and node dissection the operative wounds healed by primary intention in 12 cases (41%) secondarily in less than 2 months in 9 cases (31%) and secondarily in 2–3 months in the remaining 8 patients. In 4 of the latter cases secondary suturing had to be done. In the group of 26 patients treated by less extensive surgery the treatment consisted in vulvectomy alone in 9 cases. Healing was obtained primarily in 6 while the wound healed secondarily in less than 2 months in two and in 2–3 months in one.

Postoperative Complications

Nine of the 29 patients treated by vulvectomy and node dissection developed postoperative oedema of one or both lower limbs. During the follow-up period the oedema proved fairly stationary but moderate without major complaints in 8 cases. One patient had massive bilateral oedema, partially disabling. Apart from this, there have been no complaints after the operation In particular no disturbances of micturition.

Within the group of 26 patients treated by less extensive surgery without node dissection operation did not leave any permanent complaints.

DISCUSSION

The uncertainty of the clinical assessment of the regional lymph nodes in this series is shown in Table III. metastases in the regional lymph nodes were histologically demonstrated in 3 out of 17 patients who were clinically assessed to have no regional metastases. Among 4 patients who were clinically assessed to have regional lymph node metastases these were only found in 2 by histological examination. Several authors have pointed out that the clinical assessment of regional lymph node involvement in this disease is uncertain and of limited value (4 6, 13) This is, in our opinion, an argument for always including nodal dissection in the primary treatment.

In an attempt to estimate the value of including dissection of the regional lymph nodes in the primary treatment as a standard procedure, we have compared the group A of 29 patients treated with total vulvectomy and regional nodal dissection with the group B of 26 patients who received less extensive surgical treatment (see p. 5) without dissection of the regional lymph nodes. These two groups are compared as regards the distribution in the T-N system (Table II) and it can be seen that the most important difference between the two groups is that group A contains four patients with clinically suspicious lymph nodes, while the group B contains only one such patient.

The results of treatment in the two groups are listed in Tables IV and V and in Fig. 2.

Table IV shows the 4 years crude survival in groups A, B and C. The results of treatment expressed in this way correspond to the results in other reported series, where the treatment consisted of more extensive surgery (2, 3 4 6, 14) and less extensive surgery (1 3 6).

Naturally a comparison of the therapeutic results in different publications is very difficult owing to the great variation in classification, selection statistical treatment and other factors.

A better expression for the results of a given treatment is achieved by introducing a correction for the deaths caused by intercurrent diseases, which have a statistical significance that is disproportionately great in a disease like cancer of the vulva, where the numbers of patients are relatively small and the average age relatively high.

Fig. 2 shows the corrected annual surviving percentages in group A and group B It can be seen that the results in group A are better every year but the difference is never statistically significant.

Table V shows that the rate of local recurrences

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RADIOTHERAPY OF CARCINOMA OF THE VULVA

A. Bäckström, F Edsmyr and H. Wicklund

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Abstract During the years 1957–1966 nineteen patients with advanced carcinoma of the vulva were selected for external radiotherapy using cobalt-60 irradiation. All patients belonged to clinical stage T4 according to UICC proposed TNM-classification of carcinoma of the vulva in 1967 No other treatment could be given in these advanced cases. Biopsies were taken and thin needle aspiration biopsies were performed from suspicious inguinal nodes. The local tumour dose was in the range 5 200 rad in 37 days to 6 900 rad in 45 days. The inguinal regions were irradiated in 11 of the 19 patients. Four of the 19 patients have symptom free survival time of more than 5 years. Six patients had cytologically verified lymph node metastases in one or both groins; two of these patients have no signs of tumour growth in the inguinal regions after over 6 years. We recommend the use of supervoltage treatment for patients with advanced tumours in the vulvar region

Carcinoma of the vulva is a rare neoplastic disease and predominantly a disease of advanced age, the mean age being over sixty years (Taussig, 1940 Berven, 1949 Lundvall, 1961 Edsmyr 1962).

Methods of treatment of this disease include surgery radiotherapy and a combination of both.

Since 1922 the standard method for treatment of carcinoma of the vulva at Radiumhemmet has been bipolar electrocoagulation of the primary tumour as described by Berven (1941) and Edsmyr (1962)

In early years patients with inoperable tumours were given orthovoltage roentgen therapy palliatively The results were very poor (Edsmyr 1962)

Stoeckel (1930) collected 126 cases treated by radiotherapy at different institutions. A five-year cure rate of 12% was achieved. Different types of irradiation techniques and radiation characteristics were used in the treatment of selected cases which were not suitable for any kind of surgery Berven (1930) reported a five-year survival rate of 13% Baud (1949) of 14 and Tod (1949) of 25%

Electron beam irradiation has been recommended by German authors, not only for advanced cases. In 1965 Schubert & Hohne reported a 44% five year survival rate in 27 patients exclusively treated by electrons delivered from a 15 MeV Betatron. Renner (1965) reports a figure of 41%

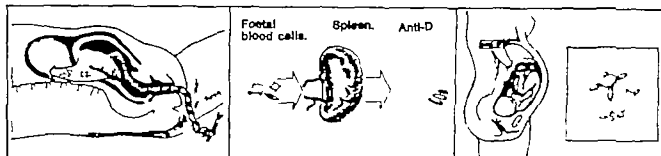
MATERIAL

The present series of 520 cases of malignant neoplasms of the vulva were examined with a view to therapy at Radiumhemmet in Stockholm during the years 1922 1966. From 1957–1966, 19 patients with advanced carcinoma of the vulva were selected for external radiotherapy using cobalt-60 irradiation. All were examined by a gynaecologist and a radiotherapist before treatment was commenced. No other treatment could be given in these advanced cases In all cases except one, biopsies were taken and examined at the Institute of Radiopathology at Karolinska Hospital. Thin-needle aspiration biopsies were performed from suspicious inguinal nodes. Evaluation and classification were uniform. The results of treatment will be presented

CLASSIFICATION

It is of great importance to have a firm clinical classification for comparison of reported therapeutic results and evaluation of different methods of therapy The Committee on Clinical Stage Classification of the UICC proposed in 1967 a T.N.M. classification of carcinoma of the vulva. The classification follows the general rules that previously have been established for carcinoma of other organs.

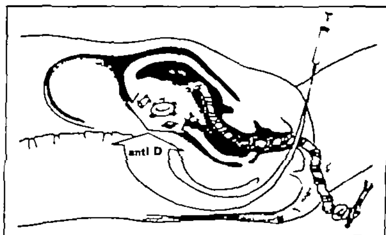
Prevention of Rh-immunization



Schematic illustration of how a small amount of blood crosses over from the foetus to the mother during parturition.

Foetal Rh-positive blood cells elicit the formation of antibodies in the Rh-negative mother

Antibodies pass over from the Rh-immunized mother to the foetus.



Prophylactic administration of Gammaglobulin anti-D to the mother within 72 hrs. after delivery in order to prevent Rh-immunization.

Gammaglobulin anti-D Kabi

without mercury Storage refrigerator Shelf life 3 years.
Pack 250µg ampoules comb package



Table I. *Patients treated by cobalt-60 teletherapy f carcinoma of the vulva*

		Primary tumour			Inguinal region					
Patient	Clinical classification	Tumour dose (rad)	Time (days)	Irradiation technique	Dose at 3.5 cm depth rad	Time (days)	Irradiation technique	Died of int. disease	Died of cancer	Alive after (years)
FKJ	T4 N0	5 400	40	Three perineal beams	—	—				<1
KLI	T4 N1	6 000	32	Moving beam	4 000	12	Overlap.			8
EG	T4 N3	6 900	45	Moving beam	4 000	10	Overlap.			$6^{9}/_{12}$
LKE	T4 N3	5 900	31	Two edge filter beams	4 000	23	Overlap.			$6^{4}/_{12}$
NEM	T4 N1	5 900	34	T wedge filter beams	1 800	7	Teleradium			<2
ORV	T4 N1	6 700	50	Two wedge filter beams	6 000 (right)	33	Telecobalt			$6^{9}/_{12}$
JT	T4 N0	5 200	37	Two wedge filter beams	—	—	—			<2
VAM	T4 N2	6 300	43	Two wedge filter beams	4 000	15	Overlap.			<1
LAE	T4 N0	6 400	44	Two wedge filter beams	—	—	—			<2
AE	T4 N1	6 000	52	Two wedge filter beams	3 500 (right) 5 000 (left)	20 31	Overlap. telecobalt			<1
NAM	T4 N0	6 000	42	Two wedge filter beams	3 500	19	Overlap.			<2
PG	T4 N0	6 200	41	One post. and two ant. wedge filter beams	4 700	41	Telecobalt			3
ÖR	T4 N1	6 100	41	Two wedge filter beams	5 800 (right) 5 900 (left)	24 29	Telecobalt			$2^{3}/_{12}$
ÖAI	T4 N0	6 000	43	Two wedge filter beams	—	—	—			<1
EGU	T4 N0	6 400	46	Two wedge filter beams	—	—	—			<1
BEK	T4 N0	6 800	70	Two wedge filter beams	—	—	—			<1
WEV	T4 N3	6 000	49	Two wedge filter beams	2 900	27	Telecobalt			<1
FK	T4 N1	5 800	48	Two wedge filter beams	—	—	—			$4^{8}/_{12}$
HNH	T4 N3	5 500	37	Two wedge filter beams		—	—			<1

24–41 days. One patient was treated by the overlapping beam technique to one groin and cobalt-60 teletherapy to the other groin. One patient received treatment to both groins with teleradium units, giving dose of approximately 1 800 rad at 3.5 cm depth in 7 days.

CASE REPORTS

The following are case reports of the patients living more than 2 years after radiotherapy.

1. 34-year-old woman (K. L. I.). Tumour infiltrating the posterior part of the left labium majora, the ischio-rectal fossa and the perineum, fixation to the pelvic wall. Biopsy revealed squamous cell carcinoma. Thin needle aspiration biopsy of clinically benign lymph node in the left groin revealed lymphadenitis.

Clinical classification: T4 N1.

The primary tumour dose was 6 000 rad in 32 days. In addition 4 000 rad at 3.5 cm depth was administered to the inguinal regions in 12 days.

There was slight primary reaction of the skin and mucous membranes. There was no recurrence of tumour, but slight telangiectases in the vulval region and slight subcutaneous fibrosis in the groins were noted at follow-up examinations. There re no bladder or intestinal disturbances. This was satisfactory qualitative result and the patient is alive 8 years after radiotherapy.

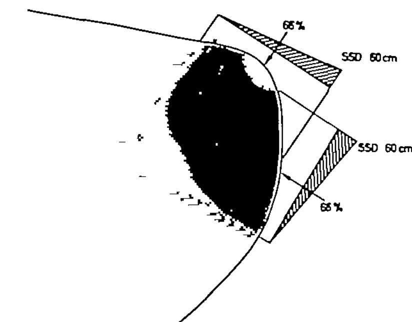


Fig 1 Diagram of dose distribution in a medial sagittal section through the pelvis using ^{60}Co SSD 60 cm, two wedge filter beams.

Carcinoma of the vulva

T Primary tumour

- T1 Single tumour not more than 2 cm in its largest dimension
- T2 Single tumour more than 2 cm, but not more than 5 cm in its largest extension.
- T3 Single tumour more than 5 cm in its greatest extension of tumour of any size with extension to vagina not more than 2 cm in length or to anal canal without involvement of mucosa, or with extension to urethra.
- T3m Multiple tumours covering an area not more than 10 cm in diameter
- T4 Single tumour of any size with extension to vagina more than 2 cm in length or to anal canal with involvement of the mucosa or to rectovaginal septum or to other neighbouring structures.
- T4m. Multiple tumours covering an area more than 10 cm in diameter

N Regional nodes.

- N0 No palpable nodes.
- N1 Movable homolateral nodes.
- N2 Movable contralateral or bilateral nodes.
- N3 Fixed nodes.

M. Distant metastases.

- M0 No distant metastases.
- M1 Distant metastases present.

All patients in this series had very advanced tumours of clinical classification T4 There was no possibility of radical surgery (vulvectomy) or any kind of exision or electrocoagulation. All patients were kept in hospital during the treatment and careful consideration was given to every kind of local or general reaction.

EXTERNAL BEAM IRRADIATION TECHNIQUE

The primary tumours were in all cases treated with cobalt-60 teletherapy In 16 cases a two-beam technique was used, employing two beams with wedge filters (Fig. 1) Moving beam technique was used in two cases. In one case a three-beam technique was used where the beams were centred in a transverse section through the lower border of the symphysid using one posterior beam and two angulated anterior beams with wedge filters.

The inguinal regions were irradiated in 11 out of the 19 patients 8 were treated bilaterally and 3 unilaterally In 5 cases irradiation of the inguinal regions was performed using the short distance overlapping beam ^{60}Co technique (Lindell & Walstam, 1956 Edsmyr & Walstam 1959). Each treatment series comprises ten square beams 6 × 6 cm, at 10 cm SSD in a row the centres of the beams were separated by 1 cm each beam exposure being 800 or 900 R (Fig 3) This gives 3 500 or 4 000 rad at 3.5 cm depth to the inguinal region in 10–13 days. In three cases cobalt-60 teletherapy was given unilaterally and in one bilaterally to the inguinal regions, administering a dose of 2 900–6 000 rad at 3.5 cm depth in

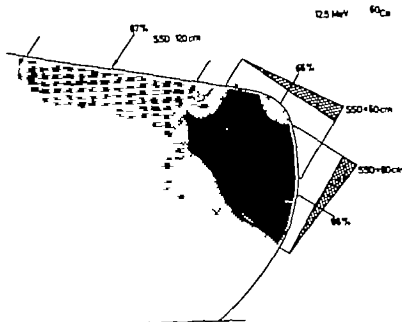


Fig. 4. The dose distribution in sections through the pelvis along *A–B–C* (Fig. 2) when ^{60}Co, SSD 60 cm, two wedge filter beams are combined with 12.5 MeV electron beam to the inguinal region.

majora, extended to the anal sphincter and was fixed to the pelvic all. Biopsy revealed squamous cell carcinoma. In the right groin, movable cytologically verified lymph node metastasis was present.

Clinical classification. T4 N1.

The primary tumour dose was 6 700 rad in 50 days. In addition, 6 000 rad at 3.5 cm depth were delivered in 33 days to the right groin.

There was moderate discomfort from intestine and bladder at the final stage of treatment. Four months later clinically residual tumour as growing, but histopathological examination of resected tumour disclosed necrotic tissue. No recurrence was observed at subsequent examinations. A qualitatively good result. The patient as alive 6 years after radiotherapy.

5. *54-year-old* oman (P. G.). A tumour involved the right labia minora and majora and extended 3 cm into the vagina, infiltrated the recto-vaginal septum and anal sphincter. Biopsy and cytological smear revealed squamous cell carcinoma. There were no palpable lymph nodes.

Clinical classification. T4 N0.

The primary tumour dose as 6 200 rad in 41 days. I addition, 4 700 rad at 3.5 cm depth were delivered to the inguinal regions in 41 days. There as slight primary reaction of the skin and mucous membrane. Slight teleangiectases developed in the vulvar region. There was no stricture of the introitus. There was no recurrence, but slight subcutaneous fibrosis occurred in the groins. A qualitatively good result, ith the patient alive 3 years after radiotherapy.

6. *7 ear-old* oman (O. E.). The tumour infiltrated the left labia minora and majora, the posterior commissure and half of the right labia minor and majora. No biopsy as performed.

I the left groin there as cytologically verified lymph node metastasis of squamous cell carcinoma.

Clinical classification: T4 N1.

The primary tumour dose was 6 100 rad in 41 days. In addition, 5 800 rad at 3.5 cm depth in 21 days on the right side and 5 900 rad in 29 days on the left side were administered.

There was pronounced local discomfort and pain from the vulvar region and also diarrhoea at the end of the treatment. The latter was relieved by instillations of solution of cortisone. The local tumour disappeared. Residual metastases in the left groin were inoperable because of the bad condition of the patient. She was alive 2 years 5 months after radiotherapy.

7. *91-year-old* woman (F. K.). The tumour extended over the posterior portions of both sides of the labia minora and majora, the posterior commissure, perineum, recto-vaginal septum and there as infiltration of the anal sphincter and anal mucosa. The tumour extended 8 cm into the vagina.

Biopsy revealed squamous cell carcinoma. There was clinically suspicious movable lymph node metastases in the right groin. Thin-needle aspiration biopsy showed lymphadenitis.

Clinical classification. T4 N1.

The primary tumour dose as 5 800 rad in 48 days.

There was slight primary reaction. Two years later cytologically verified lymph node metastasis was detected in the left groin. This as removed and histopathologically verified as squamous cell carcinoma. The patient is alive and ithout symptoms 3 years 2 months after radiotherapy.

RESULTS AND DISCUSSION

Nineteen women with locally ery advanced carcinoma of the vulva, clinical classification T4 were treated by cobalt-60 teletherapy.

Fig 2 The dose distributions in Figs. 3 and 4 are valid for sections through line A–B–C The section through AB represents the inguinal lymph node region, the section through BC is a sagittal section. The dose plan is normalized to give 100±5% to the primary tumour An electron beam is applied to the left inguinal region (cf. Fig. 4), and to the right an application of the short distance overlapping technique (cf. Fig. 3).

2. 64-year-old omen (E. O) An egg-sized tumour was present on the labium majora, growing into the vagina and posterior commissure A primarily local excision of the tumour was performed but after 1 month there was serious recurrence in the rear left of the agina, the labia majora and the posterior commissure Biopsy revealed squamous cell carcinoma. In the medial part of the left groin there was a cytologically verified fixed lymph node metastasis.

Clinical classification: T4 N3.

The primary tumour dose was 6 900 rad in 45 days. In addition, 4 000 rad at 3.5 cm depth was delivered to the inguinal regions in 10 days.

No recurrence was observed but there were slight teleangiectases in the vulval region and slight subcutaneous fibrosis in the groins. There was no stricture of the introitus. This was a qualitatively good result. The patient died of cirrhosis of the liver 6 years 9 months after radiotherapy N signs of cancer were observed at subsequent autopsy

3 59-year-old woman (L. K. E.). An ulcerating tumour, 5×3 cm was infiltrating the rear portion of the right labia majora, the perineum, tissues and the recto-vaginal septum. Biopsy revealed squamous cell carcinoma. A fixed, clinically palpable lymph node metastasis was present in the right groin.

Clinical classification: T4 N3

The primary tumour dose was 5 800 rad in 31 days. In addition 4 000 rad at 3.5 cm depth was delivered to the inguinal regions in 23 days.

There was very slight primary skin and mucous reaction. Four months later the lymph node metastasis in the right groin was removed through unilateral groin dissection. Histopathological diagnosis revealed squamous cell carcinoma.

A satisfactory qualitatively good result with slight oedema and teleangiectases together with slight atrophy of the vulvar region. The patient was alive 6 years 4 months after radiotherapy

4 53-year-old omen (O R. V). An egg-sized, ulcerated tumour infiltrated the right posterior portion of the labia

Fig 3 The dose distribution in sections through the pelvis along A–B–C (cf Fig. 2) when ^{60}Co, SSD 60 cm, two edge filter beams are combined with ^{60}Co short distance overlapping beam technique to the lymph region.

to the primary tumour treatment technique—than the previous overlapping beam technique—it has been decided to use the electron technique for the inguinal regions in the future. The general shape of the electron beams is given by available beam collimators but it can easily be modified by applying a plate of lead a few mm thick over areas to be shielded.

For patients with very advanced tumour in the vulvar region we recommend the use of super voltage treatment. The primary results in our selected series have been satisfactory with 4 of 19 patients having a *symptom-free* survival time of more than 5 years.

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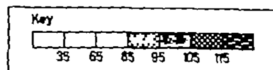


Fig 5 Key of dose levels to figures 1-4.

Different irradiation techniques were used (Figs. 1-4) The doses to the primary tumour was 5 200-6 900 rad in 31-70 days. In 8 patients the inguinal regions were not treated, while in the other 11 the short distance overlapping beam technique or cobalt-60 teletherapy or a combination were applied. The dose at a depth of 3.5 cm was estimated to 2 900-6 000 rad in 10-41 days.

Four of the 19 patients have a symptom-free survival time of more than 5 years. They received tumour doses of 5 800-6 900 rad in 31-50 days (see Table I)

Six patients had cytologically verified lymph node metastasis in one or both groins. Two of these patients have no signs of tumour growth in the inguinal regions after 6 years 9 months and 6 years 4 months respectively The tumour doses at a depth of 3.5 cm were 4 000 rad in respectively 10 and 23 days.

The primary reactions were very slight in all patients during the treatment and late reactions were few The calculated average dose to the primary tumour for each patient has been plotted in a dose versus treatment time diagram (Fig. 5) Patients living more than 5 years are all found above the line indicating healing of squamous cell carcinoma of the skin according to Strandqvist (1944) Ten of the 12 patients who died of cancer are found under the line The error in determination of the "calculated average tumour dose" is estimated to $\pm 10\%$ and includes errors in the measurements of the output from the treatment units, the three-dimensional dose distribution, mobility of organs within the patient, possible change of body contour during the treatment and inaccuracy in patient positioning.

Different irradiation techniques were used for the treatment of the primary tumour The dose distribution of the cobalt-60 teletherapy using two angulated wedge filter beams in the sagittal plane *(Fig. 1) shows a good homogeneity in the target* tumour volume. It also gives a lower volume dose than the technique using three beams arranged in a horizontal section The patients are irradiated in the lithotomy position and the alignment of the beams is fairly easy

When irradiating the inguinal regions with the short-distance overlapping beam technique, it is very important to avoid over or underdosage in the tissue volume between the primary tumour and the inguinal regions. Careful drawings must be made and the borders of the two treatment regions properly adapted to each other

The dose distribution in the section *ABC* in Fig. 2 is illustrated in Fig. 3 when irradiation of the primary tumour and the inguinal region is performed according to techniques described above

A fairly homogeneous dose distribution is obtained in the borderline area. The dose to the inguinal lymph nodes will be approximately 65% of the dose given to the primary tumour in the vulvar region.

An alternative irradiation technique is also shown in Fig. 4 An electron beam of 12.5 MeV is applied to the inguinal region and combined with the two cobalt-60 wedge filter beams to the vulvar region. By careful consideration of the borderline problems, a good and acceptable dose distribution is also achieved here

Since it is easier to adapt the electron beam

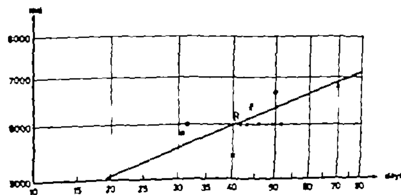


Fig 6. Dose to treatment-time relation diagram. ● indicate patients living >5 years; ○ patients living <5 years; patients dead from cancer than 5 years. —— indicates the healing f squamous cell carcinoma of the skin according to Strandqvist (1944).

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TISSUE PROGESTERONE ASSAY BY MEANS OF COMPETITIVE PROTEIN BINDING

Ingrid Nilsson

From the Department of Obstetrics and Gynaecology University Hospital (Head, Lars Ph. Bengtsson), Lund, Sweden

Abstract. An assay technique is described which permits the estimation of nanogram amounts of progesterone in tissues. After primary extraction of the tissue homogenate, the extract is purified by means of thin layer and column chromatography. Progesterone is then measured by competitive protein binding. With this technique it is possible to determine the amount of progesterone in less than 1 gram of myometrial or endometrial tissue.

During the last years improved methods have permitted determination of progesterone in tissues with high progesterone concentration, e.g. the placenta (Zander et al., 1958 Wiest, 1967) or in tissues from which large samples can be obtained, e.g. the myometrium (Barnes et al., 1962 Kumar & Barnes, 1965 Zander & Runnebaum, 1967 Wiest, 1967 Runnebaum & Zander 1971). Most investigators have used gas chromatographic techniques

With the introduction of competitive protein binding it became possible to determine very small amounts of progesterone. Plasma progesterone assays permitting determination of nanogram amounts in small plasma volumes have been described among others by Neill et al. (1967) Johansson (1969), Reeves et al. (1970) and Stone et al. (1971).

One of the most important target tissues for progesterone is the endometrium. Due to a low progesterone content in endometrium, obtained from an ordinary curettage (below 50 ng in the secretory phase) none of the techniques used hitherto is sensitive enough to determine progesterone concentration in endometrial samples from non-pregnant women

The aim of the present study was to improve the purification of the tissue extracts with special respect to specificity to make possible the use of protein binding technique for progesterone determination in 0.2–0.5 g of endometrium or similarly small samples of other tissues.

The following abbreviations will be used. CBG corticosteroid binding globulin CPB competitive protein binding GLC, gas liquid chromatography MO methoxylamine and TLC, thin layer chromatography

MATERIAL

Chemicals and reagents

Petrol ether (bp 30–60°C, Mallinckrodt) and dimethyl formamide (Mallinckrodt) were used as purchased. Ether ethanol, ethyl acetate, methanol, benzene, pyridine acetic anhydride, heptane and chloroform were purified according to Bush (1961) and distilled. Water was freshly distilled in glass before use for the binding solution. Krebs-Ringer bicarbonate solution.

Florisil (60–100 mesh, Floridin Co.) was washed ten times with distilled water to remove fines. It was dried at 100°C over night and kept in a dispenser as described by de Souza et al. (1970)

Corticosterone, 1,2-H^3 and Progesterone -4-C^{14} were from NEN Chemicals GmbH, Frankfurt/Main, Germany

Plasma for the binding solution was drawn from healthy women taking combined oestrogen/progestogen contraceptive pills. 100 μl of this plasma was used to 100 ml of binding solution

Eastman chromagram sheet 20 20 cm No. 6060 (silica gel with fluorescent indicator) were washed twice in spectrographically pure methanol and activated at 80°C for 15 min before use.

Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) was allowed to swell for at least 3 hours in the solvent heptane/chloroform/ethanol 200 200 1, saturated with water as described by Murphy (1970). It was packed in 30 1 cm glass column with glass fibre plate no. G 1 inserted at the bottom of the column. Running rate was about 20 ml/h. The progesterone fraction was collected between 12–20 ml.

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With the introduction of competitive protein binding it became possible to determine very small amounts of progesterone. Plasma progesterone assays permitting determination of nanogram amounts in small plasma volumes have been described among others by Neill et al. (1967), Johansson (1969), Reeves et al. (1970) and Stone et al. (1971).

One of the most important target tissues for progesterone is the endometrium. Due to a low progesterone content in endometrium, obtained from an ordinary curettage (below 50 ng in the secretory phase) none of the techniques used hitherto is sensitive enough to determine progesterone concentration in endometrial samples from non-pregnant women.

The aim of the present study was to improve the purification of the tissue extracts with special respect to specificity to make possible the use of protein binding technique for progesterone determination in 0.2–0.5 g of endometrium or similarly small samples of other tissues.

The following abbreviations will be used: CBG corticosteroid binding globulin, CPB competitive protein binding, GLC, gas liquid chromatography, MO methoxylamine and TLC, thin layer chromatography.

MATERIAL

Chemicals and reagents

Petrol ether (bp 30–60°C, Mallinckrodt) and dimethyl formamide (Mallinckrodt) were used as purchased. Ether, ethanol, ethyl acetate, methanol, benzene, pyridine, acetic anhydride, heptane and chloroform were purified according to Bush (1961) and distilled. Water was freshly distilled in glass before use for the binding solution. Krebs-Ringer bicarbonate solution.

Florisil (60–100 mesh, Floridin Co.) was washed ten times with distilled water to remove fines. It was dried at 100°C over night and kept in a desiccator as described by de Souza et al. (1970).

Corticosterone, 1,2-^{3}H and Progesterone -4-C^{14} were from NEN Chemicals GmbH, Frankfurt/Main, Germany.

Plasma for the binding solution was drawn from healthy women taking combined oestrogen/progestogen contraceptive pills. 100 µl of this plasma was used to 100 ml of binding solution.

Eastman chromagram sheet 20×20 cm No. 6060 (silica gel with fluorescent indicator) were washed twice in spectrographically pure methanol and activated at 80°C for 15 min before use.

Sephadex LH 20 (Pharmacia Fine Chemicals, Uppsala, Sweden) was allowed to swell for at least 3 hours in the solvent heptane/chloroform/ethanol 200:200:1, saturated with water as described by Murphy (1970). It was packed in a 30×1 cm glass column with glass filter plate no. G 1 inserted at the bottom of the column. Running rate was about 20 ml/h. The progesterone fraction was collected between 12–20 ml.

Table I *Progesterone µg/g placenta determined by different methods*

	GLC SE 30 200°C	GLC XE-60 220°C	GLC SE 30 MO 215°C	UV MeOH 240 nm	UV H_2SO 295 nm	CPB
A	2.79	2.87				2.70
B	2.57	2.52				2.74
C	48	2.67				2.58
D			3 13			2.67
E				2.80		
F					2.86	

Apparatus

GLC was performed with a Perkin-Elmer 801 apparatus with a flame ionization detector on columns 3% SE-30 and 3% XE-60. Six-feet glass columns with i.d. / " were used. The optical density was measured in a Perkin-Elmer Hitachi Spectrophotometer UV Vis Model 139

Liquid scintillation counting was performed in a Packard Liquid Scintillation Spectrometer model 33 0 using 0.7% PPO, 0 03 POPOP and 10% naphtalene in dioxane.

Tissues

Placenta was taken from a full term normal vaginal delivery

Myometrial samples were taken from full term caesarean sections and from a hysterectomy in the 11th week of pregnancy

Endometrial samples were taken during different phases of the menstrual cycle and at caesarean sections.

METHOD

The tissue samples were immediat ly put into cold Krebs-Ringer solution, thoroughly rinsed from blood and cut into small pieces. The homogenization and primary extraction was done according to Runnebaum & Zander (1971). After homogenization with distilled water 5 olumes of ether ethanol 1 3 was added and the homogenization was continued for 5 min After centrifugation the residue was washed twice w th ether ethanol and the combined extracts were evaporated t 40° t volume of 1–2 ml. It was transferred to tube w th 1 ml of water and shaken by hand with 3 4 ml of ethyl acetate The xtracts were transferred to centrifuge tube and evaporated to dryness. F ve ml of 70% methanol was added and the tube was kept in 40 water bath to dissolve the residue Then 5 ml more of methanol wa added and the sample was left t – 18 over-night. It was thereafter centrifuged at – 15 C for 20 min t 5 000 rpm. The supernatant was transferred to test tube and evaporated to 3 ml and was then tracted four times with 5 ml of petrol ther The latter was evaporated and the extract was purified on thin layer in the system ether benzene 1 After elutio from the thin layer the sample was acetylated with 50 µl f acetic anhydride in 50 µl of pyridine over-night. It was then run in TLC system ether/dimethyl formamide 99 1 Pure progesterone was run on each TLC sheet and after visualization under an ultraviolet lamp the area corresponding to the standard progesterone spot was eluted on the bent needle of a syringe. The extract was chromatographed on Sephadex LH 20 in the heptane/chloroform ethanol system and appropriate parts of the eluate were taken to CPB assay A standard curve consisting of duplicates of 0.1–0.–0.5–1 0–.0–5 0–10.0 ng of progesterone was run with each series and was plotted in semilogarithmic diagram. One ml f the binding solution was added to each tube and the rack was shaken for 5 min at 45 C in a water-bath. The samples were then cooled for 70 min in an ice-bath and occasionally shaken. 80 mg of Florisil was added to one tube at time and shaken in a Vortex mixer for exactly 30 sec and then returned to the ice bath. The time interval between the Florisil shaking of each tube was rigorously controlled. When all tubes ere shaken, 0.5 ml of the supernatant was pipetted into the counting vial and again the time interval as strictly controlled so that the Florisil was in contact ith the solution for exactly the same time

RESULTS

Recovery

A About 1 500 cpm of C^{14}-progesterone was added to tissue homogenates. The recovery was measured on the eluate from the second thin layer sheet. The recovery was independent of the amount of tissue in the range 0 1–1 0 g. In eight experiments the recovery was 80.3% ± 8.3%

B Recovery from the Sephadex column was measured separately with cold progesterone to avoid contamination of the gel. The recovery of 10 ng–5 µg was 100% ± 2%

Reliability

A placenta was treated as described under Method The extract was divided into six parts (A–F) and progesterone was determined by dif ferent methods.

A–C. GLC was performed on columns SE 30 and XE-60 with oestriol triacetate as the internal standard on both columns.

D One part was treated with methoxylamine-HCL in pyridine over the night. Progesterone-3 20-diMO was determined by GLC on a 3 % SE 30 column with cholestanone as the internal standard.

E. The extract was dissolved in methanol and its UV absorption was measured at 40 .25 and 255 nm.

F The extract was dissolved in conc. H_2SO_4

and its UV absorption was measured at 250 295 and 320 nm.

The results are presented in Table I.

Myometrium

Five samples of myometrium were analysed for progesterone (Table II). Nos. 1–3 were from full term caesarean sections. Nos. 4 and 5 were taken from hysterectomy in the 11th week of pregnancy. Tissue No. 4 was from the placental site and No. 5 from the antiplacental site.

Endometrium

Ten endometrial specimens were taken at caesarean section, legal abortion and curettage during different phases of the menstrual cycle (Table III).

DISCUSSION

The main problem in CPB-analysis is that of specificity as pointed out by Reeves (1970). Using silica gel sheets he recommends the TLC system ether benzene 2 1 which in combination with the assay with CBG gives no interference from 52 investigated plasma steroids at physiological levels. Our preliminary studies on placental extracts showed that additional purification was needed for tissues. In order to get better purification we ha e added some further steps. 1) Acetylation of the extract. 2) TLC in the system ether dimethyl formamide 99 1. This system showed to leave clean eluates especially when the sample was acetylated before chromatography. 3) Column chromatography on Sephadex LH-20, suggested by Murphy (1970) not only gives cleaner extracts but also reduces the blank problem and the contribution from method interfering factors.

Runnebaum & Zander (1971) have presented a study on progesterone in the myometrium. However in their method ten times the amount of tissue is needed as compared with the present method. Our esults from myometrial samples all lie on the lower limit given by these authors.

Even after washing the tissue in Krebs-Ringer bicarbonate solution contains some blod. Presuming that the washed endometrium contains as much as 10% blood and that this blood holds the maximum concentration of the peripheral vein blood, the contribution from blood will be only 3–8% which is within the error of the method.

The progesterone concentration in different types of endometrium (Table III) suggests—although the number is small—interesting differences between early pregnancy late pregnancy proliferative phase and secretory phase.

The study is going on, and further data on endometrial progesterone concentration, elated to blood progesterone concentration and clinical data, will be presented in a forthcoming paper.

T ble II. *Progesterone concentration in the myometrium (see text)*

No	Week of pregnancy	Weight of tissue (g)	Progesterone (ng/g tissue)
1	40	2 56	47
2	40	0 51	98
3	40	0 36	73
4	11	7 86*	207
5	11	6 74*	47

* 1/30 of the original extract was sufficient for 3–5 duplicate determinations

Table III. *Progesterone concentration in the endometrium*

No	Type of endometrium	Weight of tissue (g)	Progesterone (ng/g tissue)
1	Decidua 11 w	1 132	589
2	Decidua 40 w	0 848	137
3	Decidua 40 w	1.233	102
4	Decidua 40 w	4.232	183
5	Decidua 40 w	0 362	80
6	Decidua 40 w	0 205	122
7	Secretory phase	0.374	48
8	Secretory phase	0 387	168
9	Proliferativ phase	0 228	31
10	Proliferative phase	0 176	25

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A HISTOLOGICAL STUDY OF THE EFFECT ON THE PLACENTA OF INTRA AMNIOTICALLY AND EXTRA AMNIOTICALLY INJECTED HYPERTONIC SALINE IN THERAPEUTIC ABORTION

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Abstract. The histological changes in the placenta after intra-amniotic injection of hypertonic saline for induction of abortion were compared with those after extra-amniotic injection. In both groups a thin zone of necrotic villi was found immediately beneath the membranes. The necrotic zone was sharply demarcated from the underlying villi, which were macroscopically intact. The decidual cells underneath Nitabuch's membrane showed extensive degenerative alterations. It is suggested that decidual changes rather than damage to trophoblast play a role in the induction of abortion. The mechanism by which these changes may lead to abortion is discussed.

The mechanism by which hypertonic saline induces abortion is not clearly understood. Various hypotheses have been proposed (for reviews see 1 and 8). Bengtsson & Csapo (2) have postulated that *intra-amniotically* injected hypertonic saline, by virtue of its necrotizing effect on the placenta, eliminates a protective mechanism normally blocking myometrial activity. They assumed that this blockade was maintained by the effect of progesterone on the myometrium. This hypothesis has been debated (for references see 7).

Abortion can be induced not only by *intra-amniotic*, but also by *extra-amniotic* injection of hypertonic saline. The interval between the injection and abortion is about 36 hours when either method is used (3). To our knowledge, there is only one report on the microscopic examination of the placenta after extra-amniotic injection (10). The study showed that no changes were visible in the placenta removed by hysterotomy 15–60 min after the injection.

The present paper deals with the results of a comparative histological study of the effect on the placenta of hypertonic saline injected by the above two methods. The study includes both aborted placentae, and placentae obtained by hysterectomy after previous intrauterine injection of hypertonic saline.

MATERIAL AND METHODS

Injection technique described by Bengtsson (1) was used. Placentae were obtained from 40 pregnant patients (15 to 20 weeks) who were given intrauterine injection of 20% NaCl-solution (10 ml per week of gestation, i.e., 150–200 ml). In 16 cases, the saline was given intra-amniotically and in 24 extra-amniotically. In 6 cases subtotal hysterectomy was performed six hours after the injection, which was intra-amniotic in 2 and extra-amniotic in 4 of these cases.

Placentae from spontaneous abortions, term deliveries, and untreated hysterotomy cases of similar gestational age (15 to 20 weeks), served as controls.

Fixation was performed in 10% formaldehyde according to the routine method for surgical specimens. Four blocks of tissue were selected from the central part of the placenta for histological examination. In cases following hysterectomy the placenta with the adherent myometrium, was processed *en bloc*. The specimens were embedded as usual in paraffin, cut, and stained with eosin-haematoxylin, PAS and by van Gieson's method. In some cases staining for fibrin was performed using Weigert's technique.

RESULTS

Intra-amniotic injection

In placentae aborted after intra-amniotic injection of hypertonic saline, the membranes were oedematous with congested vessels, which sometimes contained thrombi. Underneath the membranes was found a narrow zone of necrotic villi, haemorrhages and fibrin deposits. The thickness of this zone varied between one tenth and one

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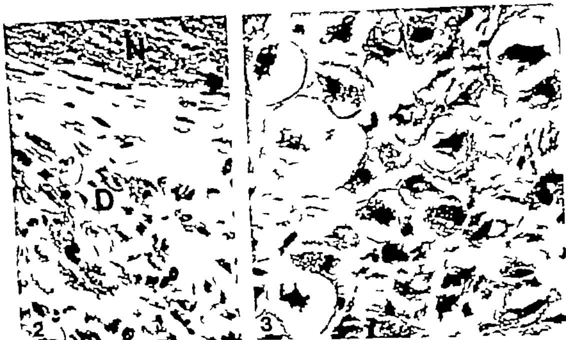


Fig. 2 Maternal part of placenta from woman treated by hysterectomy six hours after extra-amniotic injection of hypertonic saline Underneath Nitabuch membrane (*N*) the decidual cells (*D*) exhibit extensive degeneration with cell-ballooning, vacuolization of the cytoplasm, and pyknotic or disintegrating nuclei. H–E, 350.

Fig. 3 Same as Fig. 2 but at higher magnification. H–E, 575

The zone is, however rather narrow probably due to dilution of the hypertonic saline in the intervillous space and to absorption through the placental venous system

Following extra-amniotic injection, the hypertonic saline rapidly penetrates through the fetal membranes into the amniotic fluid (11 18) The intra-amniotic concentration achieved, however is lower than after direct intra-amniotic injection (12) which may explain why the necrotic zone is somewhat thinner after this type of injection

The decidual cell damage, in spite of the fact that the basal villi look microscopically normal, is surprising If the saline concentration in the basal part of placenta is high enough to induce damage of the decidual cells without injuring the basal villi, it can be attributed to a greater vulnerability of the decidual cells as compared with the trophoblast An alternative possibility is that the saline reaches the decidua basalis from the extra-amniotic space This possibility is obvious when the extra-amniotic method is used. However it is not inconceivable also with the intra-amniotic method, since hypertonic saline penetrates rapidly through the fetal membranes (11 12, 18) and accordingly may act from the extra-amniotic space.

The hypothesis of "progesterone withdrawal" as the cause of abortion (2) is based essentially on the finding of subchorionic necrosis of villi after intra-amniotic injection of hypertonic saline (4). That such an injection causes necrosis of the villi in the fetal part of the placenta has been confirmed by various investigators (6, 10 13 19–22) though some of them (6, 22) believe that the changes are far too limited to have any definite effect on the placental hormone production. The histological changes of the placenta observed in the present study were confined to a fairly narrow zone of necrotic villi immediately beneath oedematous membranes. In the major part of the placenta, the villi were of normal appearance although finer lesions, not visualized by the light microscope, cannot be excluded. Electron microscopic (22) and immunofluorescent (6) studies, however did not reveal any damage of the trophoblast in this part of the placenta after intra-amniotic injection. This together with the failure

Fig 1 Placenta aborted after *intra-amniotic* injection of hypertonic saline Adjacent to the membranes (*M*) a zone of damaged tissue (*D*) sharply demarcated from the underlying microscopically intact villi (*V*), can be seen. H E, 30

fifth of the total thickness of the placenta. The zone was sharply demarcated from the underlying villi which were microscopically intact (Fig. 1) Fibrin-like deposits were frequently seen along the lining of the intervillous space. The layer of decidual cells underneath Nitabuch's membrane showed advanced degenerative alterations with pyknotic or disintegrating cell nuclei and extensive vacuolization and dissolution of the cell cytoplasm

In spite of the fact that, in the cases terminated by hysterectomy the hypertonic saline acted for only six hours, there were no significant differences between the placentae from these patients and those from the rest of the group It was especially noted that the decidual cell changes were of the same magnitude as in the aborted placentae (Figs. 2 and 3). The myometrium in these cases was of normal histological appearance.

Extra-amniotic injection

Of the 34 women who were injected extra-amniotically 10 reported a sensation of warmth and dizziness during the injection, i.e. symptoms suggesting intravascular injection. One of these had a hysterectomy Since the histological findings in these cases differed, they are discussed separately

In placentae aborted after uncomplicated extra-amniotic injection the zone of damaged villi was slightly thinner than after intra-amniotic injection. However changes in decidua of an appearance similar to those after intra-amniotic injection were noted. There was no significant difference between placentae from the hysterectomy cases and the aborted placentae In the myometrium of the hysterectomy specimens no histological changes could be observed (Fig. 4)

The placentae from five of the ten patients with symptoms of intravascular injection did not show damaged villi which suggests that in these cases the entire amount of saline had been injected intravenously In the remaining five cases apparently only a part of the saline might have been injected intravenously since a thin zone of necrotic villi was demonstrable In the woman who had a hysterectomy after symptoms of intravascular injection the placental site showed massive infiltration of inflammatory cells, both in the decidual part and in the underlying superficial part of the myometrium. This would suggest that the saline was deposited retroplacentally

Control material

The control group (described in Material and Methods) showed no necrosis of the villi. However placentae from spontaneous abortion and term deliveries showed advanced regressive changes in decidual cells underneath Nitabuch's membrane Placentae from untreated hysterotomy cases did not show such changes.

DISCUSSION

After *intra-amniotic* injection, the fetal part of the placenta is directly exposed to the hypertonic saline which explains the observed necrotic zone.

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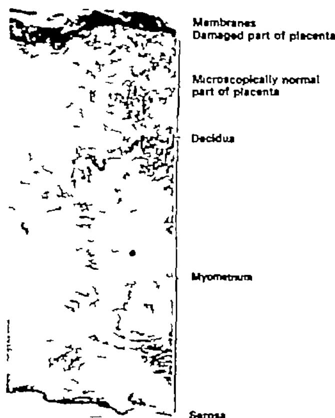


Fig 4 Section through the placenta and the uterine wall. The tissue was obtained by hysterectomy six hours after *extra-amniotic* injection of hypertonic saline. Only a narrow zone of the placenta adjacent to the membranes shows necrosis and signs of bleeding. H–E, × ___

of several workers to demonstrate a decrease of the plasma-progesterone level during saline-induced abortions (9) raises the question whether the damage of trophoblastic cells can depress the progesterone production sufficiently to result in abortion.

Regressive changes in the decidual cells could be demonstrated in all placentae except those from the hysterotomy cases in the control group. It is well known that decidual changes occur in placentae from spontaneous abortion (for references see 17) and term deliveries (for references see 16). However most reports of histological examination of the placenta after intrauterine injection of hypertonic saline, fail to mention the decidua (6, 10 13 18 19 21 2_). Strauss (20) has briefly commented that "the decidua shows occasional necrosis and haemorrhage at the edge of the placenta").

Our findings of extensive decidual cell necroses induced by the hypertonic saline may be of importance in understanding the mechanism of abortion. It is not inconceivable that this mechanism involves a release from damaged decidual cells of a substance(s) with an excitatory effect on the myometrium or alternatively by the withdrawal of a substance(s) normally produced by the decidual cells and having inhibitory action on the myometrium. Certain observations reported in the literature are worth mentioning in this respect. In patients who were not in labour Karim (14, 15) found a relatively high concentration of prostaglandin E_1 in the decidual cells, but little in the amniotic fluid and nothing in the villi. On the other hand, he found an increased amount of prostaglandins in amniotic fluid from patients in labour and also in amniotic fluid during spontaneous abortion. Since abortions can be induced by prostaglandins (for references see 5) the mode of action of hypertonic saline may possibly be a release of this compound from the damaged decidual cells resulting in abortive contractions. Studies to investigate this possibility are in progress.

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MECHANICS OF HUMAN MYOMETRIUM STUDIED WITH DYNAMIC TESTS IN VIVO

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Abstract A technique for dynamic studies of the me chanics of the uterine musculature in vivo is described, based on the registration of pressure responses to sinusoidally imposed changes in volume. The final amplitudes are varied between 1 and 9 ml and the frequency of volume changes selected between 0.005 and 0.1 Hz. Having defined the frequency of the spontaneous activity of the uterus, end pressure, area of hysteresis and distance between the limbs of the loop are used as parameters to characterize the mechanical properties of the myometrium. The potential of the method includes the study of the effect upon the myometrium of pharmacologic agents with oxytocic and relaxant effects.

Several assignments are imposed upon the human uterine wall. During growth and development of the conceptus there is, for example, an enormous increase in the olume of the uterine cavity. The myometrium adapts to this without any substantial increase in pressure. Phasic and rhythmic contractions lead to delivery of the fetus and emptying of the uterine sac. This tissue activity involves muscular changes as well as changes of the ground substance. Similar but less marked phenomena characterize the behaviour of the hollow organ in the non-pregnant woman. Anatomic changes in the muscle cells and biochemical changes in the ground substance contribute to the alterations in the pattern of spontaneous myometrial activity and intrauterine volume that occur sequentially during the ovulatory menstrual cycle.

The aim of the present work has been to find criteria in mechanical terms for the properties of the human myometrium by the registration in vivo of force-response diagrams, the hormonal and nervous control of the uterus being undis- [illegible] wall of the irregularly shaped uterine cavity is thick and architectonically complex, pressure in the cavity will be given simply as a datum in mmHg without specifying the direc tion of tension in particular portions of the uterine wall. The analysis is based on an interpretation of the shapes of curves, representing pressure re sponses during a cycle of enforced myometrial distension and relaxation. As the uterine wall con stitutes a visco-elastic system, the pressure at a given olume during the period of inflation will differ from that during deflation. The curve describes a loop, where the area between the ascend ing and descending limbs represents the combined hysteresis of the uterine wall and the re cording system.

MATERIAL AND METHODS

The dynamic response of the uterine musculature was registered in 6 volunteers. Four of the women ere completely healthy and free from gynecologic disturbances. I one of these, recordings were made during the proliferative phase, in one during the secretory phase and in one during the first day of ovulatory menstruation. The fourth woman was post-menopausal, two years having passed since her last menstruation. The fifth oman visited the clinic because of dysmenorrhea; recordings were made on two occasions on the first day of her menstruation, high spinal analgesia being administered half an hour before the second recording. The sixth woman was pregnant; she was admitted to the clinic 6 weeks after her last period, to undergo abortion.

The phase of the menstrual cycle as verified from daily determinations of basal body temperature and, at intervals, measurements of the size of the external os, calculations of the pyknotic index of the cells in the spinal smears, and determinations of the urinary excretion of estrone and estradiol as well as estriol and, in the latter part of the cycle, of pregnanediol.

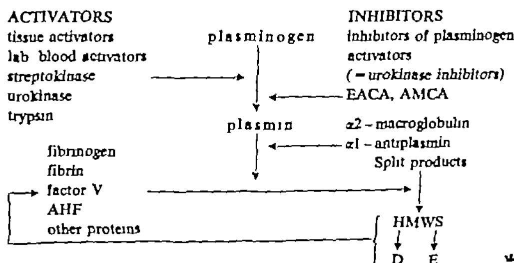
A thin-walled latex balloon as constructed so that it

Gynaecologists

Menorrhagia may be caused by an increase in local fibrinolytic activity Cyklokapron reduces menorrhagic haemorrhages by an average of 50 %

Women with average menstrual blood losses of over 80 ml have higher concentrations of plasminogen activators in the endometrium than those with lower blood losses. The resultant increase in local fibrinolytic activity is inhibited by Cyklokapron The recommended dosage of Cyklokapron in menorrhagia is 1 g 3–6 times daily for 3–6 days. With a dosage of 3 g daily Nilsson and Rybo noted reductions in bleeding of 38 % compared with control cycles. With twice this dosage bleeding was reduced by 51 None of the 36 patients participating in the tri were obliged to discontinue treatment as a res of side-effects

Reference NILSSON L., RYBO G Trea ment of menorrhagia with an antifibrinolyt agent, tranexamic acid (AMCA) A double blir investigation. Am. J Obstet. Gynecol 110 (197 p 113



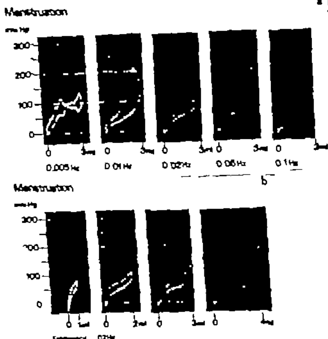


Fig. 2. Hysteresis loops recorded during the first day of ovulatory menstruation. (*a*) Amplitude of distension maintained at 3 ml; frequency of filling and emptying the receptor balloon varied between 0.005 and 0.1 Hz. (*b*) Amplitudes of muscular distension 1 ml to 4 ml; frequency of muscular distension and relaxation maintained at 0.02 Hz.

tivity of the uterus. Increasing frequencies are not accompanied by any significant additional increase in final pressure. There is no commensurate relationship between the area of hysteresis and the frequency. With successive increase in the final amplitude of muscular distension at a constant frequency (0.05 Hz and 0.02 Hz respectively) there is a progressive rise in end pressure and an increase in the area of hysteresis. In the proliferative but not in the secretory phase there is a widening of the gap between the ascending and descending limbs of the loop in proportion to the final amplitude.

Compared with the recordings during menstruation as well as with those during the proliferative and secretory phases of fertile women, the hysteresis loops recorded postmenopausally display marked monotony even when the frequency of distension and relaxation cycles is varied between 0.005 and 0.10 Hz (Fig. 5*a*). No spontaneous activity is discernible. Employing a frequency of 0.01 Hz and varying the magnitude of final muscular distension elicits a pronounced effect upon the end pressure (Fig. 5*b*). The area of the hysteresis increases but not the distance between the ascending and descending limbs of the loop. The shape of the curve changes from sigmoid figure to become more like one arch of a cubic or semicubic parabola.

Recordings of the muscular hysteresis during pregnancy (6 weeks after the onset of the last menstruation) yield curves of another character. Changing the frequency of filling and emptying the receptor balloon, using a small final magnitude of distension (7 ml) does not lead to any appreciable variation in end pressure, size of area of hysteresis, or distance between the limbs of the loop (Fig. 6*a*). Employing a frequency of 0.02 Hz for volume variation, an increase in volume from 1 to 9 ml causes the end pressure to change by a factor of 2 (Fig. 6*b*). The increase in pressure is not, however, a function of the amplitude of distension. The size of the area of hysteresis, but not the distance between the limbs of the loop, increases in proportion to the amplitude. In all cases the final part of the curve assumes a course parallel to the *X*-axis.

Recordings were also made during the first day of menstruation in a woman who attended the clinic because of severe and regularly incapacitating dysmenorrhea (Figs. 7*a* and *b*). The pressure-volume curves are irregular. Spontaneous activity is superimposed upon the limbs of the loops obtained with frequencies up to 0.1 Hz. Even when

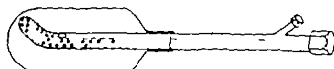


Fig. 1. The uterine receptor. A thin-walled latex balloon is glued onto twin-channel brass sound. The channel ending in the central position within the lumen is connected to the pressure transducer. The channel ending in a number of perforations is used for filling and emptying the receptor with fluid.

could be tightly attached to a twin-channel brass cannula. One channel ended in a central position within the lumen of the balloon and was always used to record pressure. The second channel, used for filling and emptying the receptor with fluid, ended inside the balloon in about twenty perforations, thereby providing a large area of contact between the sound channel and the receptor (Fig. 1).

The pressure within the receptor balloon was determined with an inductance-type pressure transducer with a 0.7 ml pressure chamber, a compliance of 3×10^{-4} mm³/mmHg and a volume elasticity of 4×10^{4x} Newton/m².

The volume in the receptor was changed and the current volume was determined by means of a 20 ml glass syringe, the cone of which was threaded into a valve sleeve with a horizontal axis, the movements of the piston being governed by an eccentric, operated by a shunt motor with variable speed transmission in the range 0 to 0.14 revolutions per sec. This sinusoidal sequence of ejection and reaspiration of fluid eliminated artefacts due to mechanical imperfections at the moment of reversal of the linear movement. The volume of liquid delivered and reaspirated by the syringe could be preset. A multiwinding linear potentiometer monitored by the movement of the syringe piston and operated by a 0–30 volt d.c. unit (accurate to within ±0.1% of the voltage applied), was used as impulse transmitter for the volume indications.

Tubes of a plastic material based on polyethylene and known to have an infinitely small modulus of elasticity were selected to connect the brass cannula channels to the transducer and the syringe. The smallest internal diameters were 1.4 mm for the pressure line and 4.0 mm for the volume line.

The signal from the pressure transducer was fed to the vertical display and the signal from the volume transducer to the horizontal display of Tektronix dual beam oscilloscope. Documentation was achieved with a Polaroid-Land camera.

Linear response for pressures from 0 to 300 mmHg was verified by static calibration of the pressure line, during which the receptor balloon was enclosed in a polymethylmetacrylate pressure chamber. The dynamic response was reliable for pressure changes with a frequency between 0 and 8 cycles per sec. The apparatus for generating changes in volume was calibrated to give linear indication of the quantity of liquid delivered in the range 0–12 ml. The dynamics of the unit for varying the volume, including the transmission line, allowed reliable documentation of the volumes actually delivered in the consecutive phases of ejection and reaspiration at frequencies far above those used in the study.

Prior to recording, the sterilized receptor unit, the brass cannula and the latex balloon were placed in the uterus without cervical dilatation. Recordings were made with the subject in a relaxed, comfortable supine position. There was always an accomodation period of 20 min before the start of recording.

The recordings were evaluated in relation to different frequencies of distension-relaxation cycles within the range 0.005–0.10 Hz as well as to different total amplitudes of distension, maximum 9 ml. The hysteresis loops run in a clockwise direction in the diagrams, with the volume indications along the horizontal axis. All the evaluations refer to so-called stable loops, obtained after three or more complete filling and emptying cycles.

RESULTS

The recordings performed on the first day of an ovulatory menstruation were arranged to represent the response in pressure as a function of a 3 ml increase in volume. The frequencies of the complete inflation and deflation cycles were within the range 0.005–0.10 Hz (Fig. 2 *a*). At a low frequency of muscular distension and relaxation the recordings were markedly influenced by the spontaneous uterine activity. At frequencies faster than 0.01 Hz, the ascending limb of the hysteresis loop showed a fast initial increase in pressure followed by a comparatively flat portion and then through a concavity by a steep increase in pressure for small increments in volume. End pressure varied between 100 and 250 mmHg. The end pressures were not proportional to the increase in frequency. The distance between the ascending and the descending limbs of the loops varied markedly. The size of the area of hysteresis did not correlate with the rate of change in volume.

Selecting a frequency of 0.02 Hz for muscular distension and relaxation, which is well within the natural range for the organ, recordings were performed for various final distension amplitudes in the range from 1 to 4 ml (Fig. 2 *b*). In the resulting series of hysteresis loops, the end pressure as well as the area of hysteresis increased proportionally to the increase in the amplitude of distension.

Examples of recordings during the proliferative and secretory phases of the menstrual cycle are given in Figs. 3 *a* and *b* and 4 *a* and *b*. Frequencies higher than 0.02 Hz are needed before the hysteresis loop is undisturbed by the spontaneous ac-

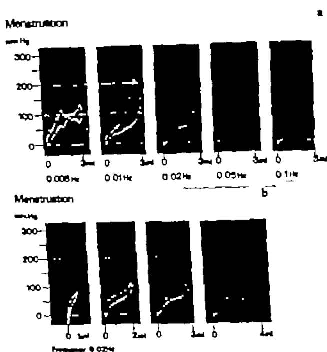


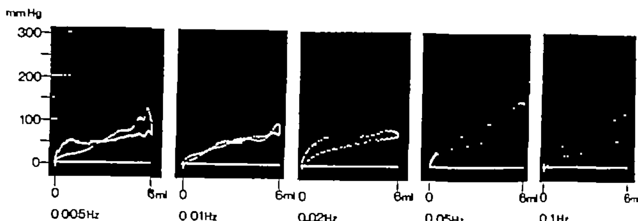
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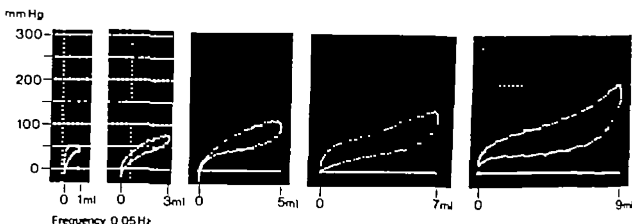


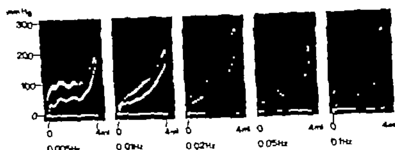
Fig 3 Examples of hysteresis loops, recorded during the proliferative phase f th. menstrual cycle. ( ) Amplitude of distension 6 ml, frequency f complete filling and emptying cycles 0.005–0.1 Hz. (*b*) Amplitudes of enforced distension bet een 1 and 9 ml frequency of the ejection-reaspiration cycles 0 05 Hz

the final amplitude is limited to 1 ml, end pressures of more than 300 mmHg are reached without any correlation with change in frequency The size of the areas of hysteresis and the distance be tween the limbs of the loops do not vary with changes in frequency but increase as a function of the amplitude of distension. The ascending limbs of the loops almost consistently display a convex course.

The picture is quite different when observations were made half an hour after the administra tion of high spinal anaesthesia (Figs. 8 *a* and *b*) The hysteresis loops are practically similar to those from ovulatory menstruation without dys menorrhea. The spontaneous activity is abolished at frequencies above 0 0? Hz. The end pressure does not reach 150 mmHg at frequencies of distension below 0 1 Hz but it is proportional to the amplitude of myometrial distension as is the area of hysteresis but not the distance between the ascending and descending limbs of the loops

DISCUSSION

Muscular hollow organs are characterized by amongst other things, the three basic properties of rhythmic contraction adaptation and tone The rhythmic contractions of an organ represent the activity that has proved most susceptible to me chanical definition notwithstanding the problem of whether or not the activity registered is true or distorted by *inadequacies in the experimental en*vironment. This objection applies in particular when the activity is recorded by means of the



b

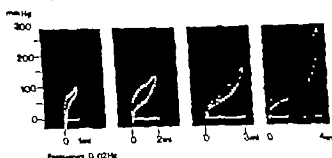


Fig. 4. Hysteresis loops recorded during the secretory phase of the menstrual cycle. (*a*) Amplitude of distension of the uterus receptor 4 ml; frequencies 0.005–0.1 Hz. (*b*) At fixed frequency of 0.02 Hz the amplitudes vary between 1 and 4 ml.

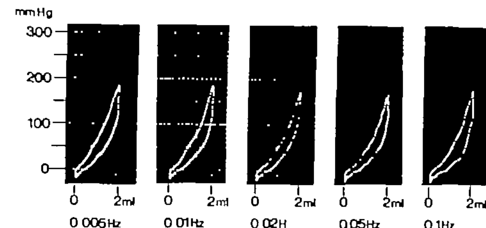
balloon technique. The method has been almost universally accepted but can be criticised on the grounds that the irritant receptor body may exercise a sensitizing action upon the myometrium.

Adaptation is an important function of smooth muscle. The term denotes the progressive elongation of the elements on mechanical loading of the structure. Adaptation dominates the behaviour of the stomach and the urinary bladder but is almost absent in tendon and is present to only a minimal degree in predominantly elastic structures such as the aorta. In physical terms, adaptability is equivalent to the phenomenon of creep or stress relaxation.

The term tone of a hollow organ is often restricted to the portion of maintained tension produced by sustained active contraction of the muscle. It has been established that both rhythmic contraction and tone are controlled by membrane depolarization mechanisms (2). If on this ground, the two processes are considered basically similar there remains the difficulty of defining tone in mechanical terms. Even though in theory the degree of tone should be indicated by comparing concurrent determinations of pressure and volume, in practice factors such as adaptation interfere and necessitate the introduction of a time scale and dynamic test systems.

From the mechanical point of view matter can be characterized by elasticity, viscosity and strength. In the present context, elasticity is the property that determines the tendency to return to an original configuration after deformation induced by an applied stress. Accordingly elasticity expresses the capacity to tolerate stress without any permanent effect. An ideal elastic element possesses strength—the property by which the material resists rupture—in addition to elasticity but has no viscosity, a property which retards deformation during stress. An ideal viscous element possesses no elasticity and neither has it any strength. If stress is applied to a purely viscous element, it undergoes progressive deformation, which is not reversed when the stress subsides. Such permanent deformation is referred to as flow while the reversible elastic deformation is called strain, often defined as $(l - l_0)/l_0$, l being the present and l_0 the initial length of the structure. Strain is dimensionless.

In the case of a hollow object, the term dis-



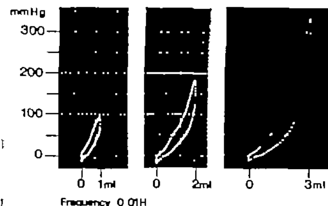


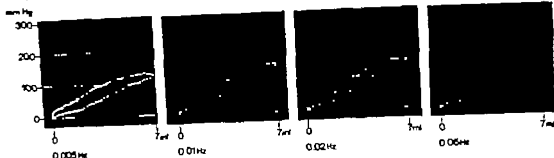
Fig 5 Recording of hysteresis during menopause. (*a*) A constant final amplitude of ml; fluid ejected and re aspirated at frequencies between 0.005 and 0.1 Hz. (*b*) Constant frequency of 0 01 Hz; amplitudes varied between 1 and 3 ml.

tensibility refers to the change in volume relative to a change in internal pressure. The curve relat ing simultaneous determinations of pressure and volume during an enforced change in one of these parameters will vary with a number of factors, the most important of which are the relation be tween the elastic and the viscous properties of the combined system of muscle fibres and ground substance the phase of registration (whether documentation takes place during distension or during relaxation) and the coincidence of the applied stress with spontaneous rhythmic activity The force response curves therefore are represented by simple pressure volume diagrams only when specifics of the recording technique suit the pur pose of the investigation. This might be the case for example when studying the variability in volume during consecutive phases of the menstrual cycle (3)

In other situations the force-response relation ship describes a loop with an area of hysteresis between the two limbs. *Parameters to be studied in* such curves are the generation of end pressure the size of the area of the hysteresis and the distance between the limbs of the loop as a func tion of changes in the frequency and amplitude of enforced distension. Also the shape of the limbs of the loop might prove to be representative of specific conditions.

In biological systems, the tissues are influenced by the presence of complicating time-dependent factors. The instability of bonds and the existence of combinations of elastic and viscous elements *necessarily make the mechanical behaviour of* muscle time-dependent. Techniques for the study of dynamic as opposed to static properties have also proved of value when investigating a tissue that is exposed to pulsatile stress influence even in the undisturbed state. An example is the clini cally valuable results, obtained at an early stage by static examination of the thin walled urinary bladder (7) Corresponding studies have been of

a



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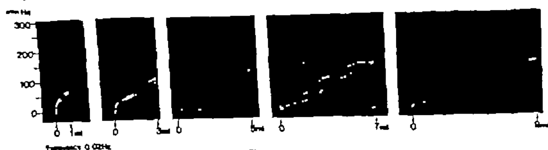
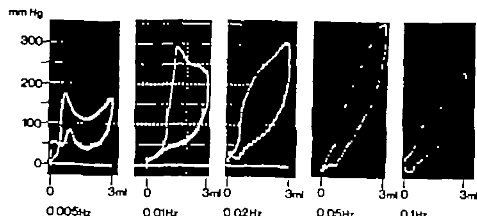


Fig. 6. Recordings during early pregnancy. (*a*) The volume added sinusoidally to the receptor within the frequency range 0.005–0.1 Hz was kept constant at 7 ml. (*b*) At frequency of 0.02 Hz, filling volumes between 1 and 9 ml were tested.

little significance, however, for characterizing the conditions in vivo of the walls of arteries. Additional problems are posed by hollow organs, such as the heart and the uterus, which have thick walls or architectonically complex muscle in an abundant ground substance. One reason for the difficulty of interpretation lies in the tendency to attribute all peculiarities in the behaviour of these organs to the activity pattern of the smooth muscle fibres only. This tendency is most probably bound up with the contention that smooth muscle cells are the only structures which can mediate the regulation of mechanical activities through neural and hormonal agents. It has been suggested recently, however, that oestrogens can provoke considerable changes even in the ground substance itself.

All the dynamic test curves obtained in this study bear witness to the hysteresis phenomenon which is due to the failure of the entire system to respond in an identical manner to the application and the withdrawal of a stress situation. The area between the limbs of the loop represents the energy that is brought to the system by the mechanical test device but not transformed into heat. The ability of the tissue to absorb energy when subjected to cyclic stress is known as the damping capacity of the tissue and it has been suggested that so-called viscous loss plays a role (1). This suggestion can, however, be challenged by the present observation that if several stretch-withdrawal cycles are recorded within a short period, the descending loop of the second and third cycles closely approximates the initial one. If lowered viscosity was of any consequence, the loops representing stretch-release should have been displaced by degrees. It was also observed that the area of hysteresis of stable loops is only seldom conditioned by the rate of stretch, as would be the case if viscous loss was of any importance. The amplitude of the applied stress had, on the other hand, a considerable influence. Smooth muscle



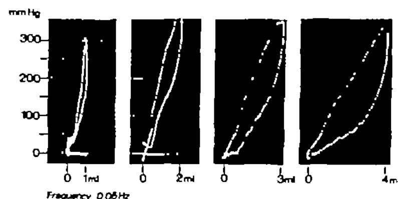
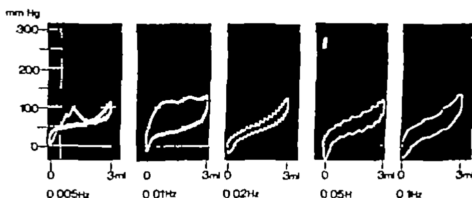
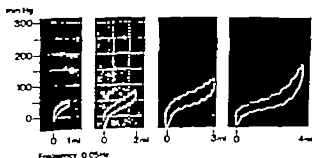


Fig 7 Hysteresis loops recorded during the first day of menstruation in oman suffering from severe dysmenorrhea. (*a*) A volume of 3 ml was ejected and reaspirated from the intrauterine receptor with cycles within the frequency range 0.005–0.1 Hz. (*b*) At a frequency of 0 05 Hz, the pressure response to volume impacts 1–4 ml as recorded.





F g 8 Hyst resis loops recorded in the same oman as in Fig. 7 30 minutes after the administration of high spinal anesthesia. ( ) Filling and emptying of the receptor with a volume of 3 ml, frequencies 0.005–0.1 Hz. (*b*) V lumes of enforced distension of the uterine cavity 1–4 ml frequency f filling and emptying cycles 0 05 *Hz*.

structures are known to be characterized by stable hysteresis on repeated stretching, without any frequency dependency (5).

Mathematical analysis of the size of the hysteresis area (including attempts to relate this to the a erage speed of instillation and reaspiration of liquid during a complete stretch-relaxation cycle) disclosed a considerable scatter even in series of recordings made at short intervals and under identical test conditions. This finding may have to do with the marked variations which were observed in the shape of the ascending loop of the curve depending upon when the stimulus, provoked by the applied distension, happened to break into the muscle's spontaneous contraction-relaxation phase. Furthermore, a consistent difference has been reported in the form of the ascending loop of hysteresis curves owing to the contracted state of the muscular organ as compared with relaxed muscular structures (6). Contracted muscle may exert its greatest influence on restricting the distensibility of the organ at small volumes. When the elongated tissue is stretched, the load shifts to the passive ground substance, which is less distensible than the muscle, and a steep pressure increase is observed at large volumes. The fact that consecutive descending loops of hysteresis curves, representing stress release, are generally super-imposable upon each other implies that the process of stretching in some way negates the contribution of the smooth muscle's contraction to the form of this limb of the loop. Therefore, among other factors, dynamic test systems that allow the application of frequencies higher than the natural frequency of the organ seem to offer advantages in addition to conventional, static pressure-transmitting techniques, which utilize intrauterine corpuscular receptors.

The effect of nervous blockade, which is seldom considered, has been observed in this study. It remains to be seen whether or not the same picture is obtained when relaxation of the uterus is achieved with pharmacologically active agents. The effect of drugs can probably be defined most suitably in mechanical terms.

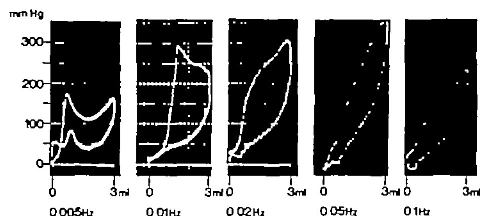
In the case of pregnant individuals, one needs to have receptor units with a considerably larger volume if the records are to provide a meaningful basis for research. Even so, the present difference in the shape of the sigmoid curve between non-pregnant and pregnant status does provide confirmation of results published recently by others (4 7).

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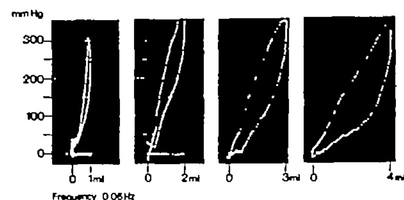
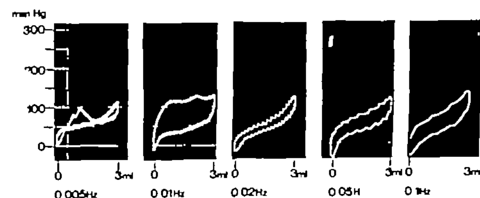


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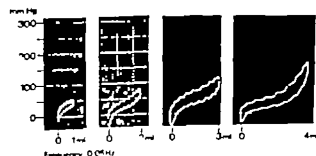


Fig 8 Hysteresis loops recorded in the same woman as in Fig. 7 30 minutes after the administration of high spinal anesthesia. (*a*) Filling and emptying of the receptor with volume of 3 ml, frequencies 0.005–0.1 Hz. (*b*) Volumes of enforced distension of the uterine cavity 1–4 ml; frequency of filling and emptying cycles 0.05 Hz.

structures are known to be characterized by stable hysteresis on repeated stretching, without any frequency dependency (5).

Mathematical analysis of the size of the hysteresis area (including attempts to relate this to the average speed of instillation and reaspiration of liquid during a complete stretch-relaxation cycle) disclosed a considerable scatter even in series of recordings made at short intervals and under identical test conditions. This finding may have to do with the marked variations which were observed in the shape of the ascending loop of the curve depending upon when the stimulus, provoked by the applied distension, happened to break into the muscle's spontaneous contraction-relaxation phase. Furthermore, a consistent difference has been reported in the form of the ascending loop of hysteresis curves owing to the contracted state of the muscular organ as compared with relaxed muscular structures (6). Contracted muscle may exert its greatest influence on restricting the distensibility of the organ at small volumes. When the elongated tissue is stretched, the load shifts to the passive ground substance, which is less distensible than the muscle, and a steep pressure increase is observed at large volumes. The fact that consecutive descending loops of hysteresis curves, representing stress release, are generally super-imposable upon each other implies that the process of stretching in some way negates the contribution of the smooth muscle's contraction to the form of this limb of the loop. Therefore, among other factors, dynamic test systems that allow the application of frequencies higher than the natural frequency of the organ, seem to offer advantages in addition to conventional, static pressure-transmitting techniques, which utilize intrauterine corpuscular receptors.

The effect of nervous blockade, which is seldom considered, has been observed in this study. It remains to be seen whether or not the same picture is obtained when relaxation of the uterus is achieved with pharmacologically active agents. The effect of drugs can probably be defined most suitably in mechanical terms.

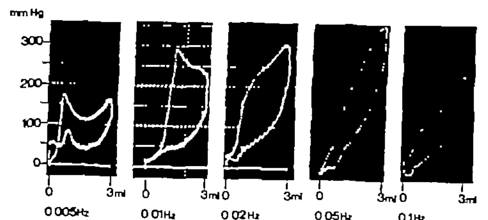
In the case of pregnant individuals, one needs to have receptor units with a considerably larger volume if the records are to provide a meaningful basis for research. Even so, the present difference in the shape of the sigmoid curve between non-pregnant and pregnant status does provide confirmation of results published recently by others (4, 7).

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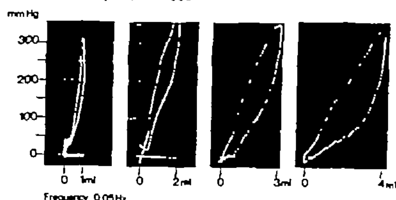
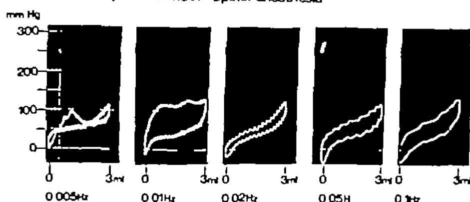


Fig 7 Hysteresis loops recorded during the first day of menstruation in a woman suffering from severe dysmenorrhea. (*a*) A volume of 3 ml was ejected and reaspirated from the intrauterine receptor with cycles within the frequency range 0 005–0 1 Hz. (*b*) At a frequency of 0.05 Hz, the pressure response to volume impacts 1–4 ml as recorded.



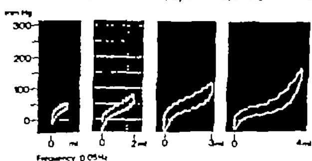


Fig 8 Hysteresis loops recorded in the same woman as in Fig. 7 30 minutes after the administration of high spinal anesthesia. ( ) Filling and emptying of the receptor with a volume of 3 ml, frequencies 0 005–0 1 Hz. (*b*) Volumes of enforced distension of the uterine cavity 1–4 ml, frequency of filling and emptying cycles 0 05 Hz.

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THE RADIOFREQUENCY FIELD DISTRIBUTION SURROUNDING COILS FOR INTRAUTERINE DIAGNOSTIC PROCEDURES

I. *Geometrical Factors*

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Abstract The absorption of radio frequency energy emitted by a small intrauterine coil has been used for intrauterine scanning in order to localize intrauterine pathology especially cancer. In order to improve the method the distribution of RF field intensities around coils of different geometries has been measured. Ringshaped coils have longer axial effective fields than other coil geometries, probably allowing diagnoses of more deeply lying processes. Still deeper processes may be reached by two-coil systems of special design. The RF absorption has been shown to follow the squared value of RF field intensity

In previous papers (1, 2, 3) it was indicated that altered radiofrequency (RF) absorption from an intrauterine coil may allow the diagnosis of endometrial cancer and other intrauterine pathology. This is due to different degrees of absorption of RF energy in various tissues because of their different biophysical properties. These properties also give rise to a shift of the resonance frequency of a tuned circuit containing the coil. In the previous papers no attention was drawn to the extension of the RF field surrounding a coil inserted into the uterine cavity. However the magnitude of the RF field at different points is of fundamental importance for the sensitivity penetration and resolution of the diagnostic procedure. Therefore, studies have been undertaken on the distribution of the RF field surrounding coils of different geometries.

THEORETICAL

(a) *Field distribution*

Suppose that a thin layer rotationally symmetric coil is used. The profile of the current carrying layer is $r = f(s)$ in the coordinate system of Fig. 1. Consider a part of the current carrier having thickness dL, the axial extension ds and the periphery length dp see Fig. 1. The width dA of this carrier element is $ds/\cos\gamma$, $\operatorname{tg}\gamma$ being defined as dr/ds. The current flowing in this small coil element is $I(ds/\cos\gamma)\,dL$, where I is current density. This current gives, at distance u, rise to an RF magnetic field vector d^3H' which is

$$d^3H' = I\,ds\,dp\,dL/u^2\cos\gamma \tag{1}$$

The field surrounding the coil is obtained if this eq. (1) is integrated over the layer thickness L, the whole periphery $2\pi r = p$ and the whole coil length, see Fig. 1.

The axial field H is related to H' by the equation $H = H'\sin\alpha$. For the axial field, outside the coil, at distance u, we find.

$$H = \int_0^L \int^{2\pi r} \int_0 \frac{\sin\alpha\, I\, ds\, dp\, dL}{u^2\cos\gamma} = 2L\pi \int_0 \frac{\sin\alpha\, I\, ds}{u^2\cos\gamma} \tag{2}$$

Here $\sin\alpha = r/u$ and $u = \sqrt{r^2 + (s+x)^2}$ so that

$$H = 2\pi L I \int \frac{r^2\, ds}{(r^2 + (s+x)^2)^{3/2}(1 + (dr/ds)^2)} \tag{3}$$

This is the equation describing the axial RF field outside a coil of any arbitrary profile. A similar equation can be set up for any para-axial field. The theory was compared with the experiment for ringformed coils (AR). For a ringformed coil the equation reduces to

(b) Two coil studies

A two-coil (or split-coil) system ATC was also studied (Fig. 2). This was used to establish a maximum of RF field at a distance from the coil outlet (Fig. 6). The coil dimensions were as follows: Ringformed coils with diameters 72 mm and 42 mm, axial distance 30 mm. The mutual inductance coupling constant was 0.15

(c) *RF absorption*

RF absorption measurements were performed with a marginal oscillator unit of the type described by Odeblad (4) working at 11.5 MHz with a thin layer absorber of a saturated KBr solution in ringformed circular coil. The coil diameter was 34 mm, with a vertical axis. The liquid absorber was a 2 mm thick layer within a glass bottle of 18 mm inner diameter. These measurements were compared with theory and with squared values of theoretical and experimentally obtained RF field intensities in Fig. 7

(*d*) The geometrical proportionality of coil data was checked on ringformed coils of various sizes, diameters 72 mm, 42 mm and 32 mm

(*e*) The frequency-inpendence of geometrical field distribution was checked on a 72 mm coil at 1.6 kHz, 16 kHz, 160 kHz.

RESULT

The results are given in Figs. 3–7. In Fig. 3 all RF amplitudes were normalized to the same value at coil outlet. From these data it is evident that the external axial field distribution varies considerably for different coil geometries.

Fig. 4 indicates a sufficient agreement between theory and experimental data. The para-axial and perpendicular fields just outside the coil outlet are presented in Fig. 5. It is evident that the para-axial field outside the coil decreases.

By the use of two-coil system with directly opposing field vectors i.e. negative mutual inductance, it has been possible to obtain nearly zero axial field at the coil outlet but a fairly high field at some distance outside the coil. The field of the coil combination used is shown in Fig. 6. The curves presented in Fig. 3 represent the fields acting on a tissue or sample in a symmetry axis of a coil. These fields are in accordance with the equations given above. The response back on the coil, follows the same curve. Therefore the response curve for the measurements is expected to be the square of this curve.

The experimental test of RF absorption is given in Fig. 7 and shows sufficient agreement between theory and measurements and indicates that the squared value of the RF-field is proportional to RF absorption. The squared-field relation allows the calculation of the average penetration depth for diagnostic procedures. This

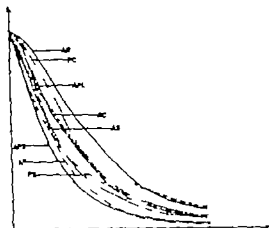


Fig. 5. Experimental data on axial and perpendicular fields for different single coil geometries. Abscissa unit = coil radius. Ordinate unit = field in coil outlet

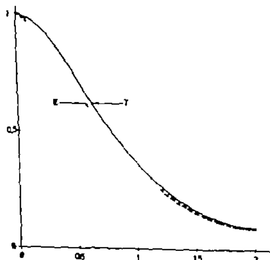


Fig. 4. Experimental *E* and theoretical *T* data on the axial RF field for ringformed coils. Units as in Fig. 3

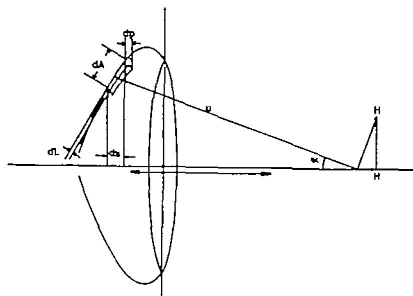


Fig 1 Schematic outline of the principles of calculation. To the left the coil of an arbitrary shape. Coil outlet at the origin of the coordinate system. The current carrying volume element is *dL dA dp* at a distance *s* from coil outlet (*s*-coordinate directed to the left from coil outlet). The field *H'* induced by the element *dL dA dp* and its axial projection *H* are also shown.

$$H = \frac{2\pi ILSr^2}{(r^2 + x^2)^{3/2}} \quad (4)$$

where ILS = current flowing in the coil ring = ampere turns. Equation (4) is shown and compared with experiment in Fig. 4

(*b*) *RF absorption and frequency shift*

These factors must be independent of RF field direction and are therefore proportional to an even potency of *H*. An experimental test of the relation between field and absorption will be reported below

EXPERIMENTAL

(*a*) *Single coil studies*

Measurements were undertaken at a frequency of 16 kHz, from a Philips GM 2305 C generator which supplied test coils at the appropriate output impedance. The field was measured with a small pick up coil. The induced RF voltage was displayed on an oscilloscope screen and its peak to-peak amplitude was measured directly on the screen

The external axial field was measured for several single coil geometries (Fig. 2)

1 Ringformed coil (AR) diameter 7? mm.

2 Cylindrical coil (AC) diameter 55 mm, length 52 mm.

3 Conical (pyramidal) coil, large aperture 39 mm, small aperture 18 mm, length 23 mm (*a*) the large aperture (APL) and (*b*) the small aperture (APS)

4 Plane (flat) coil (AF) outer diameter 53 mm, inner diameter 15 mm.

5 Spherical coil (AS) diameter 60 mm, aperture 27 mm.

The external perpendicular field was measured for

1 Cylindrical coil (PC) diameter 55 mm, length 52 mm,

2 Spherical coil (PS) diameter 60 mm.

The notations within brackets refer to Fig. 3 in which the experimental data are given. Unit distance on the abscissa = coil radius for cases AR, AC, AS PS and PC. The largest radius is taken as unit distance for APL, APS and AF. The RF field at coil outlet was taken as unit field.

Para-axial and perpendicular fields were measured outside ringformed coils in order to establish the para-axial field vectors (Fig. 5)

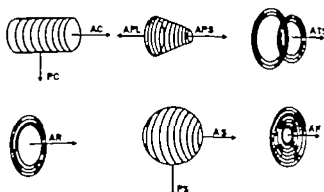


Fig 2. External axial and external perpendicular field measured for different coil geometries.

In other forthcoming papers, data on the physical chemical and histological basis for RF absorption diagnosis will be presented as well as clinical applications with refined techniques.

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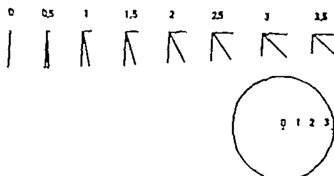


Fig 5 Para-axial field vectors experimentally measured 15 mm from coil outlet on a ringformed coil with 77 mm diameter

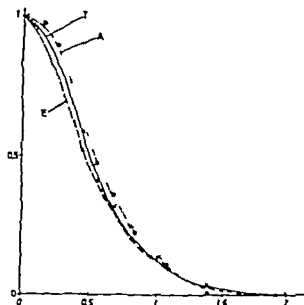


Fig 7 Experimental (*E*) and theoretical (*T*) values of squared RF field data for ringformed coil compared with experimental (*A*) RF absorption data (crosses and circles) on a KBr solution. Units as in Fig. 3

has been calculated by graphical integration and is given in Table I in units of coil radius. It follows for single coils that the penetration depth is best for a ringshaped coil. It is also evident that the two-coil system is far superior as regards depth penetration than any of the single coils.

DISCUSSION

The measurements of the field distributions have been undertaken in air and in a frequency range considerably below that used in the previous clinical trials. This situation has been chosen because stray fields and stray conduction of RF energy are then much less likely to occur and the coil geometrical effects are clearly displayed. This implies, however that the results presented in this paper are not directly applicable to the situation in vivo. Here stray conduction mechanisms occur conducting RF to deeper regions in tissues than what is indicated in the present experiments. Experiments on this problem will be reported in coming papers.

The previous clinical pilot experiments (1 – 3) were all performed using the geometry of type PC. As shown in this work the geometry is effective in measuring RF absorption only in a very thin layer probably amounting to only a few mm in the actual case. If a somewhat deeper penetration depth is desired the axial field of a ringformed coil should be used.

For still deeper penetration tests two-coil (split coil) systems should be used. Studies of these systems will be reported in coming papers. The resolution of RF diagnostic procedures has not been studied here, but will also be considered later and the same also applies to the sensitivity

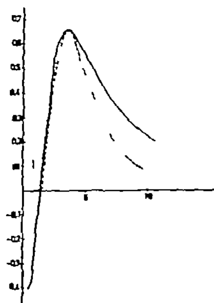


Fig 6 The field of a two-coil system ATC described in text. The RF field (solid line) maximum was at distance of 35 mm from the outlet of the small coil. The dotted line is the squared field. Abscissa in cm from coil outlet.

Table I *Penetration depth for various coil geometries*

Coil radius	unity
AR	0.35
PC	0.30
APS	0.15
ATC	1 1

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BLOOD LOSS DURING VACUUM ASPIRATION IN PRIMIGRAVID WOMEN

N. G. Holmberg and B. Sandström

From the Department of Obstetrics and Gynecology (Head: Professor P. Lundström), University of Umeå, Sweden

Abstract. Legal abortion by means of vacuum aspiration has been carried out in 78 primigravid women. The amount of blood lost per operation has been estimated. The mean hemoglobin loss during aspiration was 18.6 g. In the 35 cases where the hemoglobin percentage of peripheral blood had been determined before operation the total blood loss during the aspiration was calculated to 175 ml. There was a significantly higher hemoglobin loss at an aspiration in the 11th week of gestation than in the 10th week. No difference was found between operations in the 12th and 13th week. As a result of the above findings, which indicate only a small risk of major blood loss, we suggest that vacuum aspiration in primigravid women might be performed as an out-patient procedure before the 11th week of gestation. In weeks 11–13 the patients should be admitted to hospital but the operation still has more advantages than extra- or intra-amniotic NaCl-injections, at least in the hands of an experienced surgeon.

Since vacuum aspiration was introduced in China in the 1950s (10) a number of reports have been published. Large series have been presented especially from Eastern Europe, with particular concern for complications such as infection, perforation, and blood loss (8, 9). The amount of blood lost during operation is, however, usually poorly defined and particulars as to the possible inclusion of foetal parts and/or amniotic fluid in the blood are lacking. Operations being carried out under general anesthesia result in a heavier loss than those performed under paracervical block (PCB) (5). Margolis & Overstreet found, in a series of vacuum aspirations up to the 13th week of gestation, an average blood loss of 25 ml if the operation was performed under PCB as compared with 66 ml in cases under general anesthesia. Vladov (8) found, in 302 patients operated upon in the first trimester under short-term anesthesia, a mean blood loss of 66 ml, which corresponds to the above figures. Vojta (9) had a blood loss of 50 ml during aspiration in the 5th–8th weeks of gestation and 100 ml in the 9th–13th weeks. The type of anesthesia is not stated. In Sweden, Edström (1) found a mean blood loss of 179 ml in operations in the first trimester. Fetal parts and amniotic fluid were included in the total volume. PCB was used routinely with or without accompanying general anesthesia.

As the different published figures for blood loss are not comparable we felt it desirable to record the real blood loss in a series of primigravid women using general anesthesia (penthothal, N_2O–O_2) the blood loss being estimated as the amount of hemoglobin lost.

MATERIAL

From Jan. 1st, 1969 to Jan. 22nd, 1971, legal abortion on psychiatric and socio-medical indications was carried out in 144 primigravid women in the first trimester (defined as the first 13 weeks of pregnancy calculated from the first day of the last menstrual period). In 78 of these operations, performed by one surgeon, the same technique was used, and so these cases were chosen for studying hemoglobin loss during the operation.

The mean age of the patients was 18.7 years (range 10–15 years). The mean duration of pregnancy at the time of vacuum aspiration was 11.5 weeks (range 8–13 weeks). *The age distribution is shown in Fig. 1.*

All women were in hospital for 3 days. An out-patient gynaecological examination was carried out 2 weeks after operation. Only in one case was infection found postoperatively. That patient had been treated for gonococcal infection during the week preceding the operation. Laparoscopy showed no signs of salpingitis.

METHODS

The vacuum aspiration was in all cases performed under general anesthesia (penthothal, N_2O–O_2) after premedication of 0.75–1.0 ml morphine-scopolamine given subcutaneously. The cervix was dilated to Hegar No. 9– dur-

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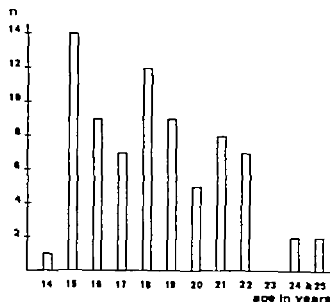


Fig 1 Age distribution of primigravid women undergoing vacuum aspiration.

ing dilatation the patient was given 1 ml Methergin® intravenously Suction tube No. 8 (Stilles – C A Nilsson 6) was inserted and a negative pressure of -0.5 kp/cm² was applied. In most cases the uterine cavity was easily emptied. If the pregnancy was of more than 11 weeks duration the cervix was dilated to Hegar 10 and suction tube No. 10 was used. The suction system was rinsed with physiological saline (300 ml) during and after the operation. No curettage was performed. The aspirated material in no case exceeded 500 ml. No operation lasted longer than 10 minutes (3)

Hemoglobin estimation

From the aspiration material several samples were taken to determine the amount of hemoglobin. The determination was carried out spectrophotometrically (Beckman DU spectrophotometer) at 544 mm (oxyhemoglobin) after adding 0.04% ammonium hydroxide. There was a good conformity between different samples from the same patient, the mean difference between the samples being ~5%. The method error of the hemoglobin determinations was calculated as 1–1.5% (4).

Table I *Loss of hemoglobin at vacuumaspiration in primigravid women in the first trimester*

Week of pregnancy	No.	Loss of Hb (g)
8	1	2.8
9	3	8.4 ± 1.8
10	11	6.4 ± 2.4
11	18	15.8 ± 6.1
12	30	24.0 ± 1_.7
13	15	23.2 ± 14.9
8–13	78	18 6 ± 12.5

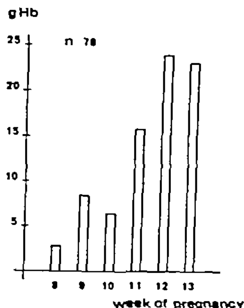


Fig 2 The hemoglobin loss during vacuum aspiration in relation to duration of pregnancy

Statistics

Means, standard deviations and variation ranges were determined according to current statistical methods (2).

RESULTS

In 35 patients the hemoglobin percentage was determined in peripheral blood before the operation, the mean being 11.5 ± 0.8 g%. When the hemoglobin quantity in the aspirated portion of the same patients was correlated to the above the blood loss could be calculated to 175 ± 108 ml.

Table I and Fig. 2 show the hemoglobin loss in relation to the duration of pregnancy in vacuum aspiration. In the 10th week of pregnancy the mean hemoglobin loss was 6.4 g and in the 11th week 15 8 g. These figures differ significantly ($p < 0.001$) In the 12th week of pregnancy the mean loss was 24 0 g, which does not differ significantly from 23.2 g found in the 13th week. The difference between the hemoglobin loss in vacuum aspirations in the 11th and 12th week is, however almost significant ($p \leq 0.05$)

DISCUSSION

Performing hemoglobin determinations on the aspirated material gives a more exact measure of the amount of blood lost than only estimating the volume of the aspirate there being no correction for the amount of amniotic fluid.

The loss of hemoglobin and volume of blood in the present investigation is larger than has been found by other workers (1, 5, 7, 8, 9). The reason for this is presumably that our material consists solely of primigravid women and that paracervical block with vasoconstrictors was not used during the operation. It seems probable that primigravid women bleed more during vacuum aspiration than multigravid women (7). This might be due to greater difficulty in dilating the cervix, with the accompanying occurrence of vascular ruptures, possibly also to a loss of uterine muscle tonus during the operation. In earlier series where paracervical block has been used either exclusively or in combination with general anesthesia, there is a higher prevalence of secondary bleeding than in our series, where there was no such case.

The rather large difference in blood loss between operations before and after the 11th week of pregnancy might be an expression of the development of the premature placenta to a more independent circulatory unit.

It seems probable that in primigravid women vacuum aspiration can be performed in the out-patient department up to the end of the 10th week of pregnancy. The operation is then relatively simple with little risk of hemorrhagic complications. From the 11th week admission to hospital might be preferable. In special cases vacuum aspiration can be carried out in the 13th week of pregnancy as an alternative to saline-induced abortion.

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URETERIC DILATATION AND RENAL CORTICAL INDEX AFTER NORMAL AND PRE ECLAMPTIC PREGNANCIES

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Abstract Ureteric dilatation was established by intravenous pyelography in the early puerperium in 78% of the 41 patients and 4–6 months post partum in 32% of the 18 patients examined. The change occurred in the early puerperium in 90% of the patients after a normal and in 67% after pre-eclamptic pregnancy. The statistically almost significant difference was attributed in pre-eclampsia to the reducing effect it exerts on uterine circulation. Dilatation was more common on the right than on the left side.

Pre-eclampsia or dilatation of the upper urinary channels did not affect the ratio of the roentgenologic area of the renal pelves and the kidneys.

The length of the kidneys for the group examined 4–6 months post partum was 0.7 cm shorter on the right and 0.7–0.9 cm shorter on the left side than for the patients investigated in the early puerperium.

Ureteric compression caused by venous dilatation from the intensified uterine blood flow during pregnancy was considered to have an important role in the etiology of ureteric dilatation in pregnancy.

Dilatation of the upper urinary tract on intravenous pyelography (2, 6) and slowing down of excretion on renography (17 19) have been observed fairly frequently during the middle and last trimester of pregnancy. In the first-mentioned studies the incidence of dilatation was 74–81%. Enlarged renal pelvis and ureter are found more frequently on the right than on the left side.

Blood flow through the kidney increases by 25% during pregnancy and the glomerular filtration rate by 50% (22). Both the increased blood flow and the general weight gain in pregnancy and retention of fluid cause enlargement of the kidneys. Thus, Bailey & Rolleston (2) reported the kidneys of puerperal patients to be an average of 1) cm longer than those of non-pregnant women.

Toxemia of pregnancy may affect renal function. Proteinuria is the most manifest indication of it. Kidney biopsies and light and electron microscopic studies have shown that pre-eclampsia causes swelling of the endothelial cells of glomerular capillaries and glomerulus capsules. Fibrin has been observed to accumulate in the endothelial cells. This causes in turn diminution of the lumen of the capillaries (12, 21). It has been established that renal blood flow and glomerular filtration are smaller in patients with pre-eclampsia than in normal gravidae (1 7). Renal blood flow has been found to be 50% smaller in severe forms of pre-eclampsia than in normal pregnancy.

The aim of the present work was to establish with intravenous pyelographic methods whether dilatation of the upper urinary tract is similar after a normal and pre-eclamptic pregnancy. The authors sought, moreover, to find out whether the dilatation of the upper urinary tract and, on the other hand, the renal circulation properties of pre-eclampsia influence the ratio between the roentgenologic area of the renal pelvis and the total area of the kidneys. The so-called renal cortical index was used in the clarification of this question (25). Its determination will be described later.

MATERIAL

The material consisted of 59 patients who had been delivered during the period August 1 1970 February 28, 1971 (Table I). The patients were divided into two groups depending on whether their pregnancy had been normal (group 1) or whether they had had pre-eclampsia during pregnancy (group 2). Intravenous pyelography was performed on the first or second post-partum day on the

Table I *Characteristics of investigation groups regarding the nature of pregnancy and time of the pyelographic examination. Means of maternal age and parity and birth weights of children and the range for duration of pregnancy by groups*

Group	N of patients	Nature of pregnancy	Time post partum of pyelography	Age	Parity	Weight of child	Duration of pregnancy (weeks)
1	20	Normal	1–2 days	25.2	—	3 500	36–40
2a	21	Pre-ecl.	1–2 days	25.7	2.3	3 340	37–40
2b	18	Pre-ecl.	4–6 months	27.3	2.6	3 170	33–40

patients of group 1. The pre-eclampsia group was distributed further into two groups according to whether intravenous pyelography post partum was performed on the 1st–2nd day after delivery (group 2a) or 4–6 months after it (group 2b). There were no significant differences between the groups in respect of the patients' age and parity and the means of the children's birth weights. The duration of pregnancy was also similar in the different groups (36–40 weeks) with the exception of one case in group 2b in which induction of labour was indicated after 33 weeks of gestation because of severe pre-eclampsia. Cesarean section was performed on one patient in both group 1 and 2a. All the other deliveries were vaginal. Nine patients in group 2b had had urinary tract infection during pregnancy.

Pre-eclampsia was classified as mild or severe according to the criteria presented in 1952 by the American Committee on Maternal Welfare (18). Of the pre-eclamptics of the patients of group 2a, 11 were severe and 10 mild, whereas in group 2b nine were severe and nine mild.

Intravenous pyelography

The patient was given an enema on the morning of the day of examination. Before pyelography the patient had neither food nor fluid for at least 3 hours. No premedication was administered.

For the investigation, 20 ml of Urografin® contrast medium was injected intravenously in approx. 70 sec. The radiographs were taken in the anterio-posterior direction with the patient supine, 5, 10 and 15 min after the injection. If the ureters were not visualised properly a further radiograph was taken 20 min after the injection. In addition to conventional radiographs, a radiograph was made about 15 min after the injection using the zonographic technique. Ureteral compression was not used in the investigations performed in early puerperium. It was applied for the radiographs taken at 5 and 10 min in the investigations carried out 4–6 months post partum.

Examination of the pyelographs

Attention was paid to the following points in the analysis of the pyelographs:

1) Dilatation of the ureters and renal pelves. The ureter was regarded as enlarged if it was of at least 7 mm breadth in the radiographs taken without compression. The borderline value between normal and pathological was estimated to be 5–7 mm by Spiro & Fry (23). If the filled ureter failed to visualise, the renal pelvis was evaluated by eye as normal or hydronephrotic.

2) The ratio between the total roentgenologic areas of the renal pelves and the kidneys. This ratio was determined by the so-called renal cortical index. This is a number expressing the fraction of the whole kidney which is occupied by the cavitary system (pelvis and calyces). The renal cortical index is calculated from the formula $(C \times D)/(A \times B)$, where A is the length and B the width of the kidney, C the length and D the width of the calyx-pelvis system.

Fig. 1a is a schematic representation of the measurement of the renal cortical index and Fig. 1b shows marked in the urograph the boundary lines used in the measuring.

The measurements were made from the radiographs taken by the zonography technique observing the double-blind principle.

RESULTS

Ureteric dilatation was established in 78% (32/41) of the patients in the early puerperium (Table II). It was more frequent after normal (group 1) than after pre-eclamptic pregnancy (group 2a). The difference was statistically almost significant ($90\% < p < 95\%$) estimated per patient. On the other hand there were no statistically significant differences when the right and left side were evaluated separately. Ureteric dilatations in the early puerperium were encountered on the right side in 28 and on the left in 14 cases. An enlarged ureter or renal pelvis was observed 4–6 months post partum in 7 of 18 patients examined. The lower limit of the ureteric dilatation was found to be at the L V–S I level in 19 cases on the right and in 9 on the left side (Figs 2 and 3). The ureteric dilatation was not seen to extend as far as the bladder in a single case. The ureter failed to fill with the contrast medium on the right side in 3 patients. The renal pelvis revealed





r 1 (*a*) A schematic drawing of the length of the axes be measured. *A B* is the (relative) area of the whole dney *C D* the area of the cavitary system. (*b*) The necessary points for measuring the axes presented in the schematic figure are marked on pyelography film.

ydronephrotic enlargement in every one of them Fig. 4).

The outlines of the kidneys and renal pelvis ould be defined in 54/59 cases on the right and 1/59 on the left side. No statistically significant ifferences in the renal cortical index alues measured from them were seen between the different groups (Table III). In the early puerper ium the renal cortical index of the patients with ureteric dilatation was greater on an verage on both sides than that of the patients with normal ureters and renal pelves (Table IV). However the differences were not statistically significant although the difference on the right was indica tive (80% < p < 90%).

No statistically significant differences were established in the early puerperium in the mean

Table II. *Dilatation of upper urinary tract in early puerperium and 4–6 months post partum*

Group	No. of patients examined	Dilatated upper urinary tract: No of patients		Right side	Left side
1	20	18	90	15	9
2	21	14	67	13	5
2b	18	7	32	7	1
Total	59	39	66	35	15



Fig. 2. Intravenous pyelography in early puerperium. The right ureter is dilatated and convoluted. Renal pelvis and calyces are normal. Unobstructed emptying on the left.



Fig 3 Intravenous pyelography in early puerperium. Renal pelvis and ureter considerably dilatated on the right. Unobstructed emptying on the left.

renal lengths in the investigations made after a normal and a pre-eclamptic pregnancy (Table V). The length of the kidney on the right side in the group examined 4–6 months post partum was 0.7 cm smaller than in the early puerperal groups. The corresponding difference for the left side was 0.7 and 0.9 mm.

Ureteric and renal pelvic dilatations were not found to be correlated with the lengths of the kidneys in early puerperium (Table VI).

DISCUSSION

The etiology of the dilatation of the upper urinary tract in pregnancy has not been clarified conclusively. Several theories have been expounded. They have attributed the dilatation to hormonal or circulatory factors or to mechanical compression by the uterus. The theory which takes into consideration the different changes during pregnancy in the circulation of the true pelvis would appear to be the most logical. Clark (8) introduced the so-called "right ovarian vein syndrome". He says: "The right ovarian vein syndrome may be defined as an aberrant ovarian vein causing right ureteral obstruction generally at the level of S-I or approximately 14 cm from the ureterovesical juncture. Ordinarily the vein causes no symptoms until it enlarges during pregnancy." In addition to Clark, Bellina and his co-workers (3) and Strohmenger & Senge (24) established operatively ureteric compression caused by ovarian veins.

The uterine blood flow is increased manifoldly during pregnancy. In consequence the capacity



Fig 4 Intravenous pyelography in early puerperium. The renal region roentgenographed by the zonography technique. *Renal pelvis* and ureter normal on the left. On the right, hydronephrotically enlarged renal pelvis and the ureter fails to fill [illegible]

Table III. *Renal cortical index in early puerperium after a normal and pre-eclamptic pregnancy and 4–6 months post partum for patients with pre-eclampsia*

Group	Right: Kidneys measured	Right: Renal cortical index, Mean	Right: Renal cortical index, Range	Left: Kidneys measured	Left: Renal cortical index, Mean	Left: Renal cortical index, Range
1	17	0 35	0.26–0.40	18	0.36	0 26–0 47
2	21	0.34	0 26–0 42	18	0.36	0 25–0 41
2b	16	0 35	0 31–0 42	15	0.37	0.31–0.42

Table IV *Ratio between width of ureter and the renal cortical index in early puerperium*

Width of ureter	Right: Kidneys measured	Right: Renal cortical index, Mean	Right: Renal cortical index, Range	Left: Kidneys measured	Left: Renal cortical index, Mean	Left: Renal cortical index, Range
Less than 7 mm	13	0 33	0 26–0 41	24	0.35	0.25–0.47
7 mm or more	25	0.35	0.26–0 40	13	0.37	0.30–0 44

Table V *Length of kidneys in early puerperium after a normal and pre-eclamptic pregnancy and 4–6 months post partum for patients with pre-eclampsia*

Group	Right: Kidneys measured	Right: Length of kidney (cm), Mean	Right: Length of kidney (cm), Range	Left: Kidneys measured	Left: Length of kidney (cm), Mean	Left: Length of kidney (cm), Range
1	17	13.6	11.9–14 6	18	14.2	12.2 15.9
2a	21	13 6	9.8–16 0	18	14 0	12.9–16 2
2b	16	12 9	11.0–14 2	15	13.3	11.2 15.5

T ble VI. *Ratio between width of ureter and length of kidney in early puerperium*

Width of ureter	Right: Kidneys measured	Right: Length of kidney (cm), Mean	Right: Length of kidney (cm), Range	Left: Kidneys measured	Left: Length of kidney (cm), Mean	Left: Length of kidney (cm), Range
Less than 7 mm	13	13.9	12.1–16 0	24	13 9	12.2 16.2
7 mm or more	25	13.5	9 8–15.8	13	14 2	13.1–15.9

of the eins in the ovarian pedicles has been increased over sixty times by the thirty-sixth week of pregnancy (13) The majority of the branches of the ovarian vein run on the ventral side of the ureter but some may pass dorsal to it (4). Dilatated veins by themselves or together with the enlarged uterus may cause mechanical compression by the uterus and result in stasis in the upper urinary channels. It is understandable from the foregoing that ureteric stasis may originate

even during the third month of gestation (14) It is possible that it could not have arisen by then solely through compression from the uterus. Furthermore the asymmetry of the change argues against both a uterine compression and hormonal etiology However the circulatory special conditions make it understandable that stasis is more common on the right than on the left side. Duré-Smith (10) reported that the right ureter is susceptible to compression for the reason that the ureter iliac artery and vein all cross at one point, and this point lies at or close to the pelvic brim In addition he noted in cadaver examinations that the right ureter curves sharply into the pelvic cavity at the same point. On the left side in contrast the crossing of the ureters and the vessels mentioned does not occur at the same point. It may justifiably be assumed, moreover that venous flow from the uterus is stronger along the right than the left veins. The thus heavily dilated veins on the right may aggravate the ureteric stasis further The following viewpoints may be propounded in support of intensified flow on the right side

1) The left common iliac vein has to pass over right iliac artery and may be compressed between the artery and the uterus during pregnancy resulting in stasis at this point. This, again, prevents outflow from the uterus via the uterine veins on the left. This viewpoint is supported by the greater frequency of varicose changes in the left leg during pregnancy

2) Uterine phlebographic studies have established that flow from the uterus occurs more frequently via the right ovarian vein which discharges direct into the caval vein than via the left ovarian vein which discharges into the caval vein through the renal vein (15 16) The venous flow during pregnancy through these vessels is obviously similarly suggestive of a preponderance of the right. The left renal vein has been found to be prone to compression when lying between the aorta and the uterus (5)

The foregoing may be summarised as follows. ureteric dilatation is caused by external compression on the ureter by dilatated veins that lead off from the uterus and by the enlarged uterus itself The high progesterone content of blood during pregnancy renders the ureters readily compressible on account of its depressive effect on the smooth musculature

In the present results, ureteric dilatation was established in the early puerperium in 90 % of the patients after a normal and 67% after a pre eclamptic pregnancy The statistically almost significant difference might perhaps be attributable also to circulatory factors. Uterine blood flow has been found to be lower in pre-eclampsia than during a normal pregnancy (21) Hence dilatation of the veins that lead off from the uterus is smaller in pre-eclampsia and they do not cause an equally pronounced ureteric compression as in normal pregnancy

It is generally assumed that a dilatated ureter normalises in the course of 2 months after delivery (14) Sidaway (20) however reported that 10% of parous women followed for 6–12 months post partum showed ureteric dilatation. The present authors own results show likewise that involution of the ureters post partum is a very slow process. Ureteric dilatation was still present 4–6 months post partum in 32 % of the patients examined Involution may also remain permanently incomplete and result in severe hydronephrosis (9)

A mechanical factor causing permanent ureteric stasis (ureteric stone prostatic hypertrophy) has been observed to cause significant elevation of the renal cortical index (26) The results now presented established no statistically significant difference between the renal cortical indices of patients with a dilatated and those with a normal ureter

This substantiates the view that ureteric compression in pregnancy is partial and temporary and does not impair renal function significantly

Enhörning & Weaver (11) supposed that the relaxation and the dilatation of the ureter during pregnancy may be regarded as a safeguard against the rise of the intrapelvic pressure caused by ureteric compression. The ureter may then act as a low pressure reservoir that empties when the compression is released. This is based on the intra ureteric pressure examinations which they made on dogs. They noted that only with an increase in frequency of contraction waves can the ureter cope with an augmented resistance

Intravenous pyelography post partum was incapable of showing that the reduced renal blood flow established in pre-eclampsia affects the size of the kidney and the ratio between the roentgenologic area of the renal pelvis and the kidney

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SERUM CERULOPLASMIN, α_1-ANTITRYPSIN, α_2-MACROGLOBULIN AND IRON AND T BINDING PROTEINS DURING HYPERTONIC SALINE INDUCED ABORTION

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Abstract Serum ceruloplasmin, α_1-antitrypsin, α_2-macroglobulin and the iron and T binding proteins were studied in six women during abortion induced by hypertonic saline. After correction of the results for total serum protein changes (15%), no significant variations in the concentrations of the proteins studied could be observed within 48 hours following saline treatment. The suitability of specific serum proteins as indicators of the welfare of the fetoplacental unit is discussed

It is known that oral contraceptives and pregnancy affect the concentrations of some specific serum proteins (2 1 8 10 13). During pregnancy these concentrations are likely to be regulated by the hormone production of the fetoplacental unit. Disturbances in its hormone synthesizing capacity may thus be reflected in the concentrations of some maternal serum proteins. It was recently shown by Clarke et al (1) that pre-eclampsia is associated with changes in certain specific serum proteins. It may be possible to use the determination of the levels of these proteins to evaluate the welfare of the fetoplacental unit

In order to study this concept and the speed of regulation of specific maternal serum protein concentrations by the fetoplacental unit, hypertonic saline was used to destroy the fetus (7) and cause decrease in the endocrine function of the placenta (2, 15) Special attention was given to simultaneous changes in serum total protein, since changes may occur in redistribution of fluids between different fluid compartments.

MATERIAL AND METHODS

Interruption of pregnancy by transabdominal, intra-amniotic hypertonic saline method was carried out in six obstetrically normal patients. The patients were young, of low parity and 14–20 weeks pregnant. As a precautionary measure for the eventuality that anaesthesia might be necessary no oral fluids were allowed for 6 hours prior to the injection. An average of 180 ml of amniotic fluid was removed and replaced with 200 ml of 20% NaCl. The infusion was given at 1 p.m. After the hypertonic saline infusion the patients were allowed to take fluids freely at least 2 000 ml per day Six blood samples were taken at −6, ±0 (time of infusion), +6 +18, +24 and +48 hr for the assessment of proteins Total proteins (11) were determined to provide a basis for calculation of changes in fluid balance caused by the hypertonic saline.

Determination of ceruloplasmin, α_1-antitrypsin and α_2-macroglobulin

Immunological determination of ceruloplasmin, α_1-antitrypsin and α_2-macroglobulin was performed by diffusion method in agarose introduced by Yokoyama & Yamakido (16) and modified by Nikoß & Salmi (9).

Thirty wells (diameter 2.4 mm) were punched in an agarose plate 10 10 cm in size and 1 mm in thickness. 3 μl of diluted serum was applied to each well. Ceruloplasmin and α_1-antitrypsin were allowed to diffuse for 30–40 min and α_2-macroglobulin for 2 hours. After this each well was tightly covered with cellulose acetate plate (14 mm in diameter), saturated with the corresponding antiserum (Behringwerke AG Marburg/Lahn, BRD). Antiserum was absorbed in the agarose plate The duration of absorption was 20 hours with α_2-macroglobulin and 4 hours with the others. Each well became surrounded by precipitation ring, with diameter proportional to the amount of protein in the sample examined The graphs for each protein, and thus the concentrations of the unknown samples, were obtained from known standards of the samples.

Determination of iron and T binding proteins

Serum total iron binding capacity (TIBC) was determined by saturating the sample with iron, removing the free iron with resin and measuring the amount of protein-bound iron (4) Serum triiodothyronine (T_3) binding capacity was determined radioimmunochemically (5)

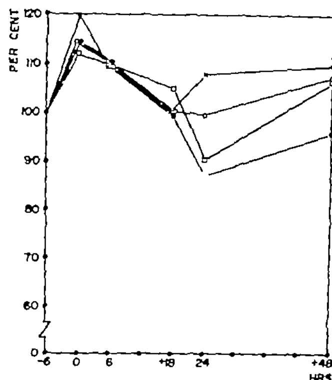


Fig. 1. Total protein, ceruloplasmin, α_1-antitrypsin and α_2-macroglobulin during six hypertonic saline-induced abortions. Hours prior (−) and after (+) 20% NaCl infusion. ●—● Total protein, ×—× ceruloplasmin, □—□ α_1-antitrypsin, ○—○ α_2-macroglobulin.

RESULTS

Fig. 1 shows that there was an increase in total protein from 7 a.m. to 1 p.m. during the morning prior to the actual procedure when the patients were taken off fluids. This was also the case with the specific proteins examined of these TIBC, α_1-antitrypsin and ceruloplasmin (Fig. 1) are given as examples. During the next 24 hours there was a fall in the values and after 48 hours the values were at the initial level.

The concentrations of the individual proteins determined were calculated to constant total protein concentration of 7 g per 100 ml. The results are shown *in Table I. It is noted that no statistically significant changes can be demonstrated during the observation period (48 hr)*

DISCUSSION

During a study like this the fetus dies within approximately 3 hours (7) the maternal plasma progesterone decreases to half the initial value (2, 15) and maternal urinary estriol excretion drops (3) On the other hand, the half-life of serum proteins is often relatively long (12) This may explain why no changes in the specific serum proteins were obtained after injury of the fetoplacental unit during the 48 hours of study. Therefore, it may well be that the possible changes in the protein concentrations determined by us are not rapid enough for clinical purposes.

The results indicate that hemoconcentration occurs prior to the initiation of saline treatment *and hemodilution after it. Compared with the initiation* of induction these changes are approximately 15% on the basis of serum total protein. Account should be taken of these hemodynamic changes when studying changes in the blood chemistry occurring in these circumstances. However the hemodynamic changes are not great enough to cause for example the decrease of 55% noted in plasma progesterone concentration (2, 15) in spite of suggestions to the contrary (14)

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Table I. *Ceruloplasmin α_1-antitrypsin α_2-macroglobulin, TIBC and T_3-test during abortion induced by hypertonic saline. Mean ± S.E.*

Hours prior to (−) and after (+) 20 NaCl infusion. Values are calculated to constant total protein of 7 g per 100 ml of serum

Hours	−6	−0	6	+18	24	48
Ceruloplasmin (mg 100 ml)	81±6.8	85±9.6	87±7.7	85±6.4	89 1	85 9.8
α_1-antitrypsin (mg 100 ml)	321±26	316±25	333±21	347±30	310±33	341 29
α_2-macroglobulin (mg 100 ml)	345±18	342±26	306±37	313±29	349 6	286 7
TIBC (μg 100 ml)	395± 1	382±17	381±18	394±20	376±18	364 19
T_3-test ( )	10.9±1.1	8.3±0.49	9.1±0.56	10.8±0.79	10.1±0.54	8.2±0.70

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ENVIRONMENTAL TEMPERATURE AND THE OCCURRENCE OF TOXAEMIA OF PREGNANCY IN A SUBARCTIC AREA

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Abstract. A study was conducted on the effect of variations of environmental temperature in subarctic area on the incidence of admission to hospital of patients with toxaemia of pregnancy. Toxaemia of pregnancy was the reason for 1 152 admissions in 1965–1968, and essential hypertension was the reason for 209 admissions. The environmental temperature was correlated with the admission rate: the higher the temperature, the greater was the number of admissions. A minor drop was seen in the rate of admissions in the temperature range 10° to + 10°C, and the same trend was observed in the frequency of the individual symptoms of toxaemia of pregnancy: oedema, proteinuria and hypertension. The incidence of eclampsia followed the same pattern, but to greater degree. The rise was higher in the temperature ranges 10° to 19°C and + 10° to + 19°C than in the 10° to + 10°C range.

Temperature changes during the two days before the day of hospital admission did not influence the frequency of hospital admissions.

Environmental temperature in subarctic area has been found to affect the incidence of for example, myocardial infarctions and cerebral vascular accidents (3 5). The perinatal mortality rate and the frequency of preterm infants and low birth weight infants are all higher in the cold winter season than in the warm summer season (4). Environmental weather conditions, strong radiation and cold weather have been observed to influence the occurrence of eclampsia, the most serious form of toxaemia (1 2).

A study was conducted at the Department of Obstetrics and Gynaecology University Hospital, Oulu, on the effect of environmental temperature and its variations on the admission of patients with toxaemia of pregnancy. Oulu is on the Gulf of Bothnia in Northern Finland, almost exactly on latitude 65 N.

MATERIALS AND METHODS

Toxaemia of pregnancy was the reason for 1 152 admissions to the Department of Obstetrics and Gynecology University Hospital, Oulu, in 1965–1968, and essential hypertension was the reason for 209 admissions. The age distribution of the patients is presented in Table I. Table II shows the distribution of the patients according to diagnostic groups. The diagnostic classification was based on the recommendation of the American Committee of Maternal Welfare. Severe pre-eclampsia was the commonest diagnostic group, followed by monosymptomatic toxaemia of pregnancy in which hypertension of pregnancy accounted for 80%. There were only 13 cases of eclampsia. Fig. 1 shows the mean monthly temperature, the monthly total of admissions and the number of patients with toxaemia of pregnancy. The figures are stated as percentages of the corresponding totals. Toxaemia of pregnancy accounted for higher proportion of the admissions in April–June than at any other time of the year. A minor peak occurred in December–January. Toxaemia of pregnancy as the reason for an average of 11%, and toxaemia of pregnancy plus essential hypertension for about 13 of the admissions in 1965–1968.

RESULTS

The effect of the daily mean temperature on admissions is evident from Table III. The mean temperatures are tabulated in groups of 10 The second plus third columns show the number and percentage of days in each temperature group. The fourth plus fifth columns giv the number of admission in each temperature range and the proportional distribution. The last column shows the calculated daily frequency of admissions. Fig. 2 shows graphically the ratio between the mean temperature and the daily distribution. When the temperature is over 0°C, the number of admissions rises in relation to the temperature.

Table I. *Distribution of patients by age*

Age (y.)	No. of patients	%
<20	185	13 6
21–25	276	20.3
26–30	248	18.2
31–35	249	18.3
36–40	248	18.2
41–45	137	10.1
>45	18	1.3

Table II *Distribution of patients by diagnostic groups*

Diagnosis	No. of patients	%
Monosymptomatic toxaemia	323	24
Mild pre-eclampsia	241	18
Severe pre-eclampsia	34	25
Eclampsia	15	1
Superimposed toxaemia	231	17
Essential hypertension	209	15
Total	1 361	100

A minor decrease in the admission frequency occurs in the temperature range −10 to +10 C, otherwise the admission frequency correlates well with the temperature on the day of admission (equation of the regression line $y = 0.0096x + 0.99$ correlation coefficient $r = +0.855$) Analysis by

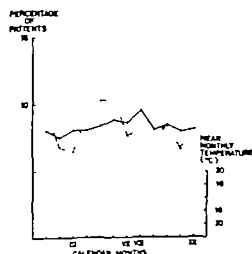


Fig 1 Mean monthly temperature, total admissions monthly and the number of patients with toxaemia of pregnancy The figures for the years *1965–1968* were combined and the monthly percentage of patients was calculated from the total. ●—● All patients O- -O toxaemia of pregnancy O- O mean temperature

Table III. *Effect of daily mean temperature on the admission of patients to hospital*

Daily temperature (C°)	Days		Admissions		
	No.	%	No.	(Incidence of total)	Per day
< −30	7	0.5	5	0.4	0.71
−29– −20	79	5 4	68	5.0	0.81
−19– −10	163	11 0	161	11.8	0.99
−9– 0	345	23 7	295	21 7	0 86
0– +9	458	31 4	409	30.1	0.89
+10– +19	394	27 0	404	29 7	1 02
> +20	15	1.0	19	1 4	1.26

diagnostic groups (Fig. 3) shows that the incidence of eclampsia is highly significantly ($p<0.001$) greater in the temperature ranges −10 to −19 C and +10 to +19 C than in the +10 to −10 C range. The change is similar to that in the total toxaemia of pregnancy series, but is *more accentuated. Gradual elevation was seen in* the daily readings on moving from −20 to +20 C in the groups with superimposed toxaemia of pregnancy and essential hypertension. No significant differences were seen in the other diagnostic groups. The incidence of the different individual symptoms of toxaemia of pregnancy such as oedema, proteinuria and hypertension, on the day of admission increased on moving from colder to warmer temperatures, but there was a decrease in the temperature range −10 to +10 C (Fig. 4) No definite differences were

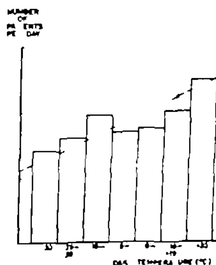


Fig Daily number of admissions in the mean temperature groups.

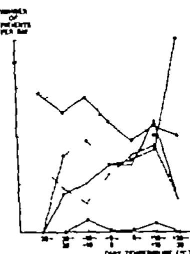


Fig. 3 Distribution of the daily number of admissions according to the diagnostic and mean temperature groups. ○—○ Monosymptomatic toxaemia of pregnancy; ○- -○, mild pre-eclampsia; ○- -○, severe pre-eclampsia; ●—● eclampsia; — superimposed toxaemia, ● essential hypertension.

observed between the temperature intervals and subjective symptoms on the day of admission, i.e. headache, visual disturbances.

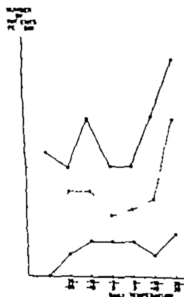


Fig. 4 Occurrence of the various symptoms of toxaemia of pregnancy daily in the different temperature groups. ○—○ Oedema, ○- -○ proteinuria; ○- -○ hypertension; ●—● subjective symptoms.

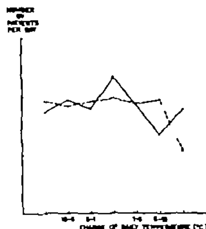


Fig. 5 The daily number of admissions in the temperature variation groups. The change of temperature was calculated for 1 day and 2 days before the day of admission. ●—● During one day; ●- -● during two days; – fall of temperature; + rise of temperature.

The effect of temperature change 1 and 2 days before the admission on the admission rate was examined. The results were expressed in so-called daily readings. The daily readings are entered (Fig. 5) on the ordinate and the temperature change groups on the abscissa. No significant differences were noted. The result is similar for the effect of temperature changes during the coldest months, January–February and the warmest, July–August (Fig. 6)

Fig. 6. The daily number of admissions in the temperature variation groups, during January–February and during July–August, calculated for 2 days before the day of admission. ●—● January–February ●- -● July–August; – fall of temperature; + rise of temperature.

CONCLUSIONS

This study made in a subarctic area shows that the environmental temperature correlates with the admission frequency of patients with toxaemia of pregnancy The higher the temperature, the greater is the number of admissions. The proportion of admissions was highest in April–May–June A minor drop was seen in the rate of admissions in the temperature range −10 to +10 C, and the same phenomenon was observed in the frequency of the individual symptoms of toxaemia of pregnancy oedema, proteinuria and hypertension. On the other hand, no such decrease was seen in the occurrence of subjective symptoms.

When the different diagnostic groups were compared with the different temperature ranges, the most accentuated effect of temperature was seen in the incidence of eclampsia. It was highly significantly greater at higher and lower temperatures than in the middle range.

Temperature changes were not found to exert a distinct effect on the frequency of hospital admissions.

On relation to toxaemia of pregnancy the most *favourable temperature range for pregnancy in a* subarctic area seems to be −10 to +10 C.

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CONSERVATIVE OPERATIVE TREATMENT OF TUBAL PREGNANCY WITH POSTOPERATIVE DAILY HYDROTUBATIONS

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Abstract. Conservative surgery in the form of incision and suture of the tube was used in the treatment of 43 tubal pregnancies. Rheomacrodex®-Hydrocortisone®-Chymotrypsin® solution was introduced into the patient's abdominal cavity at operation to prevent the formation of adhesions. Using a little modified solution, daily hydrotubations via a Foley catheter placed in the uterine cavity were performed during the period in hospital. At the follow-up examination 24 of the 32 patients subjected to salpingography had completely patent tubes. There were 26 pregnancies (60%) within the follow-up period of 4–40 months. Six of the 10 patients with only one remaining tube became pregnant during the follow-up period, and five children were born of these pregnancies. These patients had three extra-uterine pregnancies. The study indicated that conservative extra-uterine therapy combined with hydrotubation therapy to prevent adhesions gives better results than conservative therapy alone as reported previously.

The recent literature reveals an interest in conservative surgical treatment of tubal pregnancy as it seems that subsequent fertility is greater than after radical salpingectomy and that the incidence of recurrent tubal pregnancies is in fact relatively small (1, 6, 7, 8, 12). Various forms of hydrotubation have been used with varying success in cases of tubal occlusion (2, 4, 5, 9, 13, 14). Reports show that following conservative surgery approximately 35–45% of the patients have a subsequent pregnancy (10, 12, 16). The incidence of pregnancy following hydrotubation in patients with tubal occlusion ranges from about 10 to 35% in individual series (2, 3, 9). At the Department of Obstetrics and Gynaecology, University of Oulu, modified hydrotubation was combined with conservative surgical management of tubal pregnancy.

MATERIAL AND METHOD

The material consisted of 43 patients with tubal pregnancy in the region of the middle or distal third of the tube. The age range was 17–36, with a mean of 26.3 years. The parity was from 0 to 5 with a mean of 0.8. Operative treatment (section and suture of the tube) (7) was performed within 6–10 weeks (average 6.8 weeks) of the start of the last menstrual period. At the time of operation careful curettage of the lumen of the tube was performed with a blunt curette after removal of the amniotic sac. The tube was closed with a single layer of atraumatic catgut. If the tube had already ruptured, only evacuation, revision and closure of the ruptured site were performed. A conservative operation of this kind was considered only when the patient wanted more children and if there seemed to be chance of sparing the tube. After the operation 200 ml of a 10% solution of Rheomacrodex® to which was added 2 g of Hydrocortisone® and 10 000 units of Chymotrypsin® were introduced into the abdominal cavity to prevent the formation of adhesions. From the first postoperative day onwards the tubes of each patient were irrigated via a Foley catheter placed in the uterine cavity injecting slowly through the tubes 20 ml of Rheomacrodex® solution, to which was added 100 mg of Hydrocortisone® and 5 000 units of Chymotrypsin®. This hydrotubation procedure was performed daily throughout the patients' stay in the hospital, generally for 8–9 days. The catheter was removed from the uterine cavity 2 hours after the irrigation. Modified hysterosalpingography was performed on 19 patients at follow-up examination, using 30% Urografin® (11), and the contrast medium was then rinsed out of the tubes with physiological saline (11). Routine hysterosalpingography was performed on 13 patients. All the patients were treated in the years 1965–1970.

RESULTS

Hysterosalpingography performed on an average of 5 months postoperatively showed that the affected tube was completely open in 24 of the 32 patients examined. Hydrosalpinx had developed in

5 cases and in 2 cases an extensive pouch of adhesions was seen around the tube the patency of the tube was unclear in 1 case.

During the follow up period of 4–40 months, 26 pregnancies were confirmed in these 43 patients. Sixteen of them terminated in normal delivery 2 in caesarean section and 4 in extra uterine tubal pregnancy (of which one was on the contralateral side) Four patients have an intra uterine pregnancy at the time of writing. Six of the 10 patients with only a single tube left became pregnant during the follow-up period, and five infants were delivered of these pregnancies in addition, 3 of these patients had an extra uterine pregnancy and two had abortions.

DISCUSSION

According to reports in the literature pregnancies are slightly more frequent after conservative management of extra-uterine pregnancy than after salpingectomy and hydrotubations as part of the treatment of tubal occlusion have also given relatively good results. We therefore decided to combine these procedures in treating tubal pregnancy The results are very good, for pregnancies occurred in 60% during the follow-up period of 4–40 months. This is approximately 10% higher than in most other reports and is a very high figure considering the extremely short follow-up period. Furthermore the patients earlier fertility was obviously relatively low since the average number of pregnancies per patient was only 0 8 The reported recurrence rate for ectopic tubal pregnancies in the same tube varies from 3 6 to 11 8% Four of the patients in our series had a second extra-uterine pregnancy which was in the same tube in three cases. Skulj and his coworkers (15) noted that 78 7% tubes were patent after conservative surgery for extra-uterine pregnancy but when they performed 3–5 hydrotubations on the 8th–15th postoperative days the tube was patent in 86.6 % They found that prevention of adhesions was the most important consideration in operations for extra-uterine pregnancy Swolin (16) used an intraperitoneal hydrocortisone application (700 mg) on every second patient with extra-uterine pregnancy and was able to show that a statistically significant decrease in adhesions occurred in the hydrocortisone group We also tried to prevent the formation of adhesions by introducing a Hydroadreson-Chymotrypsin-Rheomacrodex solution into the patient's abdominal cavity A modified solution was used later for daily hydrotubations during the patient's hospital stay After roentgenography of the tube the contrast medium was rinsed out.

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FIBRINOLYTIC ACTIVITY DURING LABOUR

Birger Åstedt

From the Coagulation Laboratory (Head, Professor Inga Marie Nilsson) and the Department of Obstetrics and Gynaecology (Head, Professor Stig Kullander), Malmö Allmänna Sjukhus, Malmö, Sweden

Abstract. The fibrinolytic activity in the blood in 60 women during labour was compared with that in 60 women at term. Both the spontaneous fibrinolytic activity and the response of fibrinolytic activity to venous occlusion were significantly higher during labour. Fibrin degradation products (FDP) (0.5–2 mg/100 ml) appeared in 37% during labour compared with 5% in the term group. No decrease was found in the inhibitors of the fibrinolytic system. It is assumed that the rise in fibrinolytic activity during labour may be due to changes in the hormonal secretion of the placenta.

The endothelium of certain vessels, especially veins, contains activators of fibrinolysis, which are continuously liberated into the blood stream and maintain its spontaneous fibrinolytic activity. It is believed that these activators play an important role in the dissolution of early fibrin deposits otherwise prone to give rise to thrombosis (20). Induction of venous stasis of the arm normally causes a marked increase in the fibrinolytic activity of the blood in the occluded vessels owing to a local increase in the release of activators of fibrinolysis from the vessel walls (20). Robertson et al. (22) have shown that the response of the local fibrinolytic activity during venous occlusion of the arms is a suitable method for estimating the ability of an individual to release activators of fibrinolysis from vessel walls.

In the later months of pregnancy the fibrinolytic activity in the blood is substantially reduced and after delivery it rapidly returns to its non-pregnant level (1, 3, 5, 14, 18, 23). Åstedt et al. (27) also found that the local increase in fibrinolytic activity following stimulation by venous occlusion, successively diminished with advancing pregnancy and that it was almost nil at the end of the third trimester. However, little is known about the release of the fibrinolytic enzyme from the vessel walls during labour.

This paper concerns the response of the local fibrinolytic activity to venous occlusion of the arms in 60 women in labour. The investigation was extended to include determinations of some components of the fibrinolytic system.

MATERIAL AND METHODS

The clinical material consisted of 60 apparently healthy women in labour within 12 hours of delivery. 60 healthy pregnant women one week before calculated term served as a control group.

The following determinations were made: plasminogen (immunological method) (10), inhibitors of plasminogen activation by urokinase (urokinase inhibitors) (21), antiplasmin (10), α_2-macroglobulin (13) and fibrinolytic degradation products (blood collected with thrombin and epsilon aminocaproic acid) (17).

Venous occlusion was produced by wrapping sphygmomanometer cuffs around both upper arms and inflating them to a level midway between the systolic and diastolic blood pressure. The stasis was maintained for 20 min. Blood samples were obtained from antecubital veins before application of the cuff and again after venous occlusion immediately before the cuff was deflated. The fibrinolytic activity was determined by testing the plasma and resuspended euglobulin precipitate immediately on unheated fibrin plates, expressed in mm² of lysis (19). The mean value of the fibrinolytic activity after venous stasis for non-pregnant women found at our laboratory is 321 mm² with a standard deviation of 119 (22).

RESULTS

The mean values found for the components of the fibrinolytic system studied are given in the table. The concentration of α_2-macroglobulins in women during labour was significantly higher

5 cases and in 2 cases an extensive pouch of adhesions *was seen around the tube· the patency* of the tube was unclear in 1 case.

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MATERIAL AND METHODS

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The following determinations were made: plasminogen (immunological method) (10), inhibitors of plasminogen activation by urokinase (urokinase inhibitors) (21), antiplasmin (10), α_2-macroglobulin (13) and fibrinolytic degradation products (blood collected with thrombin and epsilon aminocaproic acid) (17).

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RESULTS

The mean values found for the components of the fibrinolytic system studied are given in the table. The concentration of α_2-macroglobulins in women during labour was significantly higher

Table I. *Mean values and S.D of fibrinolytic inhibitors and of plasminogen in 60 pregnant women at term and 60 in labour*

Fibrinolytic component	At term	In labour
α_2-macroglobulin, %	119 ±27	140* ±34
Antiplasmin, ACU/ml	779 ±196	851 ±190
Urokinase inhibitors, %	83 ±33	78 ±23
Plasminogen, %	149 ±42	163 ±41

* $p < 0.01$

than in the term group No significant changes were found in the values noted for the other components.

The spontaneous fibrinolytic activity in the 60 women examined during delivery was significantly ($p < 0.01$) higher than that in the 60 women at term (Fig. 1) Fibrin degradation products (FDP) (0.5–2 mg/100 ml) were demonstrated in 22 (37%) of the patients, compared with 3 (5%) in the term group The response of the local fibrinolytic activity to venous occlusion was also significantly increased ($p < 0.01$) (Fig. 2) No correlation was found between the fibrinolytic activity and the progress of labour

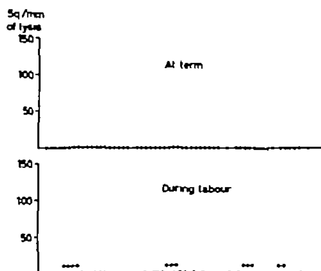


Fig 1 Spontaneous fibrinolytic activity at term and during labour (resuspended euglobulin precipitate, Sq mm of lysis on fibrin plates). Each dot denotes one patient.

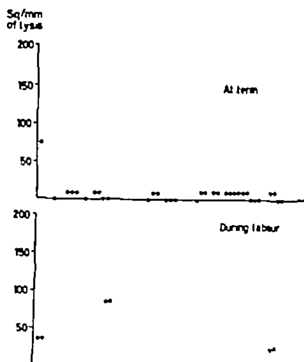


Fig 2 Response of fibrinolytic activity to venous occlusion of arms at term and during labour (resuspended euglobulin precipitate. Sq mm of lysis on fibrin plates). Each dot denotes one patient.

DISCUSSION

The spontaneous fibrinolytic activity during labour has been found not to differ from that before (1 3 4 24) These observations were made with clot lysis assays. In the present investigation however resuspended euglobulin precipitate was tested on fibrin plates and proved to be significantly higher during labour than at term. It should also be pointed out that Shaper et al. (24) compared their labour group with a pregnant group including women who had been pregnant for only 20 weeks. At such a relatively early stage of pregnancy the fibrinolytic activity is only slightly reduced (28) In the present investigation the material consisted of a pregnant group of women who were calculated to deliver within about a week, i.e. at a time when the fibrinolytic activity is markedly reduced and often barely demonstrable at all Further like the findings in this study Bonnar et al. (4) noted an increased amount of fibrin degradation products in serum of patients in labour which corroborates our observation that the spontaneous fibrinolytic activity in the blood increases during labour

This is the first time that the fibrinolytic re

sponse to venous occlusion has been studied during labour. In this investigation the fibrinolytic activity stimulated by venous occlusion was still low but significantly higher than in women at term. The response of the fibrinolytic activity to venous occlusion was thus in accord with the above mentioned rise in the spontaneous fibrinolytic activity.

The demonstration of a higher spontaneous fibrinolytic activity and of the increased release of fibrinolytic activators following stimulation by venous occlusion during labour compared with what was found in women at term might be ascribed to a decreased inhibitory effect on the fibrinolytic system. But since these inhibitors tended, if anything, to be increased during labour this suggestion may be questioned. Some other explanation must therefore be sought for the higher fibrinolytic activity during labour. It is known that the injection of adrenaline, or the influence of exercise and mental stress increase the fibrinolytic activity in the blood (2, 6, 7, 8, 12, 16, 20, 25, 26). It is therefore tempting to assume that the strenuous physical and mental stress during labour may be a contributing cause of the rise of the fibrinolytic activity in the blood.

But the higher fibrinolytic activity during labour may also be explained in another way. Many authors feel that it is an activator of the fibrinolytic system that decreases during pregnancy (4, 5, 15, 18, 24). Åstedt et al. (27) found a reduced content of fibrinolytic activators in the venous vessel wall during pregnancy and a successive reduction of the release of the activator into the blood stream. Sex hormones are believed to be involved in this mechanism (15, 24, 27). Åstedt (28) found that administration of ethinyloestradiol was followed by a significant decrease of the activator content of the vessel wall and that administration of medroxyprogesterone tended to inhibit the response of fibrinolytic activity to venous stasis (Åstedt et al. unpubl. data).

During pregnancy the placenta produces large amounts of oestrogen, progesterone and human placental lactogen. El-Tomi et al. (11) reported a decrease of human plasma placental lactogen during labour reflecting the relative uterine and placental ischaemia of labour. Opinions differ about whether there are any important changes in the high pregnant levels of oestrogens and progesterone during delivery (for ref. see 11). Recently Csapo et al. (9) have found that the plasma progesterone level falls slightly with the onset of clinical labour. It is probable that the higher level of fibrinolytic activity during labour is due to changes in the hormonal secretion of the placenta.

ACKNOWLEDGEMENT

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THE DISTRIBUTION OF ^{125}I-MARKED BOVINE PROLACTIN AND HUMAN CHORIONIC GONADOTROPHIN IN RATS WITH EXPERIMENTAL OVARIAN TUMOURS

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From the Department of Obstetrics and Gynecology Malmö General Hospital (Head, Prof Stig Kullander) and the Radiation Physics Department in Lund (Head, Prof Kurt Lidén), University of Lund, Sweden

Abstract The distribution and fate of ^{125}I-labelled bovine prolactin, human chorionic gonadotrophin and bovine serum albumin was studied and followed by serial gamma camera photographs, scintillation scanning of blood and tumors, and autoradiography in normal pro-oestrus rats, in oophorectomized rats and in rats with experimental ovarian tumours developed in intrasplenic ovarian grafts after bilateral oophorectomy A high affinity for ^{125}I-HCG was found both in ovarian tumours and in normal ovaries. An increased active tumour uptake of ^{125}I-HCG was observed for 3 hours, which permitted tumour depicting with gamma-camera technique. The ^{125}I-prolactin uptake was more moderate both in ovaries and ovarian tumours. ^{125}I-albumin showed no specific affinity The possible application of these findings in diagnosis and therapy of human ovarian tumours is discussed.

Ovarian tumours, mostly granulosa cell tumours or luteomas, easily develop in ovarian tissue grafted into the spleen of oophorectomized rats. Hypophyseal gonadotrophins stimulate the growth and the production of hormones, mainly progesterone in these ovarian tumours The same type of tumours also occur spontaneously in the rat ovary (6) That a similar gonadotrophin dependence can occur in certain human ovarian tumours is suggested by *in vitro* observations in tissue cultures of ovarian cancers (7). We therefore thought it of interest to follow the fate and distribution of ^{125}I-marked gonadotrophins introduced into the blood-stream of rats bearing intrasplenic ovarian tumours. If a concentration of radioactivity in the rat ovarian tumours was found, a similar approach might permit *in vivo* scanning of certain ovarian tumours in the clinic.

Having no access to species-specific rat gonadotrophins we labelled with ^{125}I readily available human chorionic gonadotrophin (Gonadex, 3 000 IU per mg, Leo Pharmaceutical Co., Hälsingborg, Sweden) and bovine prolactin (NIH-P B-2, mean relative potency 19 9 IU per mg) For control ^{125}I-labelled bovine serum albumin was used and normal rats, in prooestrus stage, according to the vaginal smears, as well as spayed animals.

MATERIAL AND METHODS

Oophorectomy was done under ether narcosis 1 2 days before the actual experiments. Ovarian tumours in the spleen were produced by the method described by Kullander (6). At the time of the experiments the tumours were about 18 months old and about 10 mm in diameter. At autopsy they showed the histological picture described by Kullander (6). The ^{125}I-labelling of the proteins was done by the modified Chloramine-T-method (8) with the following minor changes:

(1) use of 0 125 M borate/HCl buffer with pH 8 4 instead of pH 7 45

2) use of twice the amount of all reacting agents, in the same volume, i.e. 2 mCi of ^{125}I instead of 1 mCi; protein 2 mg/ml, 50 μg;

3) air instead of N_2.

In order to study the distribution of the radioactivity in the rats we used scintillation camera system consisting of basic unit (Nuclear Chicago Pho/Gamma III) together with 4096 channel memory scintigram analyser, an image digitizer and tape memory

A 4000 channel lead collimator was placed in front of the gammadetector This collimator is designed for gamma rays of relatively low energies. It gives the best combination of sensitivity (in terms of dots per minute recorded on the picture per μCi of activity in the subject) and resolution for this experiment (1). The high sensitivity makes it possible to study the dynamic processes in the

Research fellow on leave from the Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, Amsterdam, Netherlands.

Table I

Case	Endocrine state	I jected with	Time of autopsy after i jection (mi)
I 1	Castrate	HCG	130
I.2	Pro-oestrus	HCG	165
I.3	Pro-oestrus	HCG	165
II 1	Tumour-bearing	HCG	165
II.2	Tumour beari g	HCG	100
II.3	Tumour bearing	HCG	220
III 1	Castrate	Albumin	135
III.2	Pro-oestrus	Albumin	205
IV 1	Castrate	Prolactin	70
IV.2	Pro-oestrus	Prolactin	165
V 1	Tumour-bearing	Prolactin	165
V.2	Tumour-bearing	Prolactin	165

activity distribution. The main disadvantage however is that the resolution becomes no better than 1 mm (9 10). This makes it difficult to separate the different organs of the rat. The problem was partly solved by using lead shields on the rat.

Every rat was injected into the jugular vein, exposed surgically under ether narcosis, with one of the ^{125}I labelled proteins in about 0.5 ml of phosphate buffered saline. The total amount of ^{125}I injected was 25–50 μCi (about 3 μg albumin and prolacti and about 13 μg HCG).

After the injection the anaesthetised rat was placed 5 cm from the collimato stretched on its back with f xed legs, and a 10 minute-picture was taken. This was repeated every half hour At every occasion one picture with lead shield and one without was taken. On the tumourbearing rats th. lead shielded everything except the tumo re-gions, on the others, the lead shielded the region cranially from the live

At arious intervals after the intra enous administration of the labelled protein the animals were killed, blood was taken by puncture of the retrobulbar venous plexus and small tissue-pieces were put in plastic tubes th ml Bouin s fixation-fluid, for one day The radioactivity of the blood and the tissue samples were measured th a 3 3 NaI (Tl) well-counter The measurement time was chosen so that the standard deviation in the counting statistics was $\leq 1\%$ The fixed tissues ere eighed, blocked in paraffin and sectioned at 5 μm. Autoradiography was done by the stripping-film method (7). The ex posure time was 9–18 days. The preparations were after wards stained with Mayer s haematoxylin and eosin

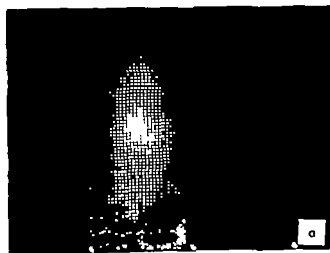
The number and type of rats (all belonging to the homozygous R-strain), injected material, and time of killing is illustrated in Table I

RESULTS

Figs. 1 and 2 are examples of the pictures received on the oscilloscope screen of the 4096 channel memory From these pictures it is possible to read the number of counts in a region of interest with a light pen We have chosen to study the activity in the heart–liver region and in the tumour region.

The mean activity in the heart–liver region as a function of the time after injection of ^{125}I labelled HCG (cases I 1 1.2, I 3 and II 1) is shown in Fig. 3 The diagram actually shows a disappearance of the activity from the blood. It is impossible to determine whether there is any active uptake in the liver during this time We have found no significant differences from Fig. 3 in the cases where prolactin or albumin was injected.

Fig. 4 shows the activity profiles in the sagittal



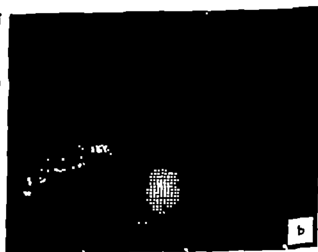


Fig 1 Photographs of the scintillation camera pictures as displa ed on the oscilloscope screen f the 4096 channel memory ( ) The activity distribution in case I 1 176–181 min after the injection (*b*) The activity distribution in case II 1, 156–161 min after the injection and with everything except the tumour region covered by lead shield



Fig. 2. The same pictures as in Fig. 1 in three dimensional display. (*a*) The main peak is from the heart–liver region. (*b*) The main peak is from the tumour.

plane of a spayed and a pro-oestrus rat (L1 and L3) injected with ^{131}I labelled HCG. The difference between the profiles indicates an active uptake in the ovaries (in the case L3). No similar difference was found in cases (III.1 and III.2) which were injected with ^{131}I labelled albumin.

The results from the measurements of the activity of the tumour regions in tumour rats (cases II.1, II.2 and II.3) injected with ^{131}I labelled HCG are shown in Figs. 5 and 6. In two cases (II.2 and II.3) the tumour was covered by the liver which made it impossible to separate the tumour from the liver on the scintillation camera pictures. However the differences (Fig. 6) against the normal curve from the heart–liver region must be due to an active uptake in the tumours. In one case (II.1) we had no such interference between the tumour and the liver and Fig. 5 clearly shows an uptake of activity in the tumour region in this rat. The conclusion must be that ovarian tumours have an active uptake of ^{131}I labelled HCG. The uptake has not reached any maximum or stabilized level 150 minutes after the injection (Fig. 5).

We have also found a tumour uptake of activity in rats injected with ^{131}I labelled prolactin (cases V.1 and V.2). Fig. 7 shows this. The uptake however is less than of HCG and reaches maximum more rapidly at approximately 40 min after the injection (Fig. 7).

Table II shows the results of the measurements of the activity in different tissues at autopsy. The ovaries show very high activities relative to the activity in the blood in pro-oestrus rats injected with ^{131}I HCG. This is in agreement with the gamma camera findings (Fig. 4) and our autoradiographic results. The radioactivity was found localized in the follicular fluid, blood-vessels and capillaries, but most concentrated, however, in the theca interna of the early corpora lutea (Fig. 8). The activity values of the ovarian tumours in rats injected with ^{131}I HCG are remarkably high,

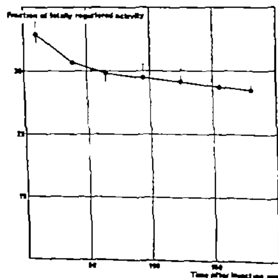


Fig. 3. The mean activity of the heart–liver region in the cases I.1, I.2, I.3 and II.1 as a function of the time after injection with ^{131}I labelled HCG. The uncertainty is given as 1 S.D.

Table I

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III.2	Pro-oestrus	Albumin	705
IV 1	Castrate	Prolactin	20
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Fig 4 shows the activity profiles in the sagittal

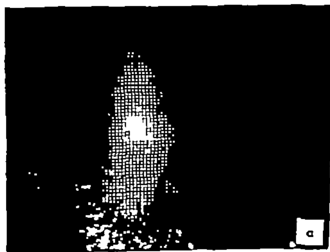




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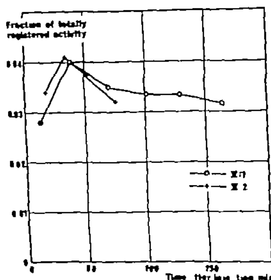


Fig. 7. The activity of the tumour regions in the cases V 1 and V 2 as function of the time after injection with ^{125}I labelled prolactin.

The value for the ovaries of a rat, injected with ^{125}I-prolactin, is only slightly higher than for a rat injected with ^{125}I-albumin. This difference would probably have been more pronounced, if we had killed the rats after 20 min. It has been found previously with a prolactin preparation labelled with ^{131}I, that the concentration of radioactivity in the ovary reaches a maximum at about 20 min after intracardiac injection and then decreases. The values at 30 min are slightly higher than for blood (11).

In the autoradiographic preparations of the ovaries of a rat (III.2) injected with ^{125}I-albumin, the localization of the radioactivity was restricted to the follicular fluid, the blood-vessels and capillaries (Figs. 9 *a* and *b*). When ^{125}I-prolactin was used (IV.2) the radioactivity was found scattered all over the corpora lutea (Fig. 10). No specific localization was found within the corpora lutea.

The activity values of the ovarian tumours of rats injected with ^{125}I-prolactin are not so high as in the normal ovaries (rat IV.2) (Table II). The histological pictures of the ovarian tumours of the rats injected with ^{125}I HCG and the rats injected with ^{125}I-prolactin were somewhat similar (Fig. 11). In the autoradiographic preparations the radioactivity was randomly distributed in the solid tumour-tissue (Fig. 12) in both cases. Some activity concentrations were seen in the blood-vessels.

DISCUSSION

Experimental ovarian tumours, developed in intrasplenic ovarian grafts in oophorectomized rats can thus be successfully scanned and depicted with gamma-camera technique after the administration of ^{125}I-HCG to the host. This is possible because of the lower uptake of the radioactive material by most other tissues. This activity of

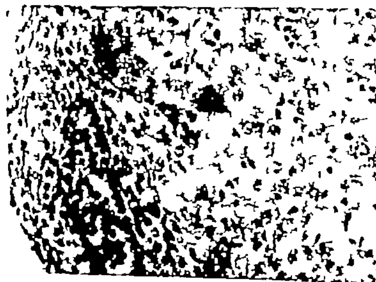


Fig. 8. Autoradiographic picture of an ovary of pro-oestrus rat injected with ^{125}I-HCG. Note high activities in the theca interna of the early corpus luteum. × 300.

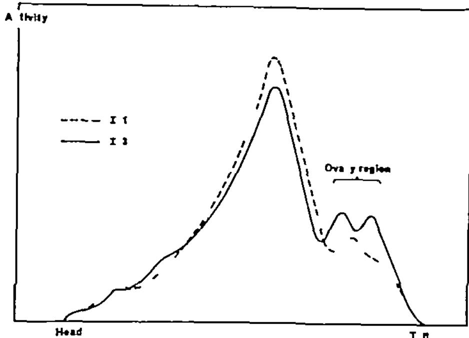


Fig 4 Activity profiles in the cases I.1 and I.3 showing an ac tive uptake of ¹²⁵I labelled HCG in the ovaries of I.3

which also agrees with our gamma camera study (Figs. 5 and 6)

No remarkable differences were seen between the values of the castrated animals and the rats in pro-oestrus stage, except in a castrated rat ((IV 1) injected with ¹²⁵I-prolactin) which had a very high value in the adrenals. This agrees with the rather high values for the adrenals of two tumour rats (V I and V.2) injected with ¹²⁵I-prolac tin. Hormonally they too may be regarded as castrated rats.

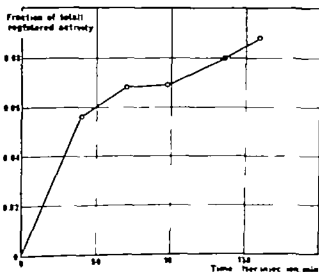


Fig 5 The activity of the tumour region in the case II 1 as a function of the time after injection with ¹²⁵I labelled HCG

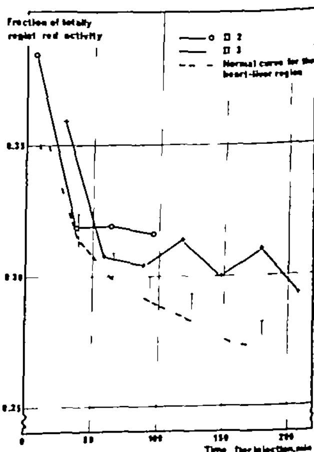


Fig 6 The activity of the heart-liver regions in the cases *II* and *II 3* function of time after injection with ¹²⁵I labelled HCG and compared with the normal case (Fig. 3)



Fig. 10 Autoradiographic picture of an ovary of pro-oestrus rat injected with ^{125}I-prolactin. The activity is randomly distributed in the corpus luteum. 920.

should be prevented by premedication with Lugol' solution. The human liver lies at a good distance from the ovaries and can be shielded.

Labelling with other isotopes should also be tested, giving possible improvements in the gamma-camera technique for this type of diagnosis with less loading and better image. The use of isotopes in this respect could also—because of the intracellular enrichment seen at autoradiography—make possible the intracellular radiation treatment of ovarian tumours

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Table II *Activities in several tissues in relation to the activity in the blood found at autopsy of the different experimental animals listed in Table I*

$\frac{\text{Activity in tissue (cpm/g)}}{\text{Activity in blood (cpm/g)}}$

Tissue	Case I 1	I 2	I 3	II 1	II 2	II 3	III 1	III 2	IV 1	IV 2	V 1	V 2
Tumour	—	—	—	1.39	1.56	1.14	—	—	—	—	0.43	0.59
Ovaries	—	3.14	12.48	—	—	—	—	0.37	—	0.75	—	—
Liver	0.58	0.64	1.02	0.83	0.61	0.86	0.57	0.20	1.18	1.02	0.66	0.40
Kidney	2.63	2.85	2.82	2.29	3.44	3.19	0.48	0.22	4.43	0.97	1.87	1.50
Spleen	0.63	0.76	0.73	0.66	0.49	0.42	0.39	0.34	0.51	0.54	0.33	0.44
Adrenal	0.34	0.68	0.62	0.53	0.68	0.54	0.58	0.47	1.31	0.61	0.70	0.71
Uterus	0.21	0.25	0.52	0.14	0.12	0.31	0.21	0.08	0.17	7.76	0.18	0.43
Hypophysis	0.30	0.24	0.52	0.19	0.45	0.45	0.54	0.26	0.39	0.27	0.19	0.16
Muscle	0.12	0.09	0.13	0.15	0.11	0.10	0.04	0.01	0.04	0.08	0.06	0.06
Fat	0.09	0.02	0.07	0.07	0.12	0.05	0.03	0.01	0.00	0.03	0.02	0.01

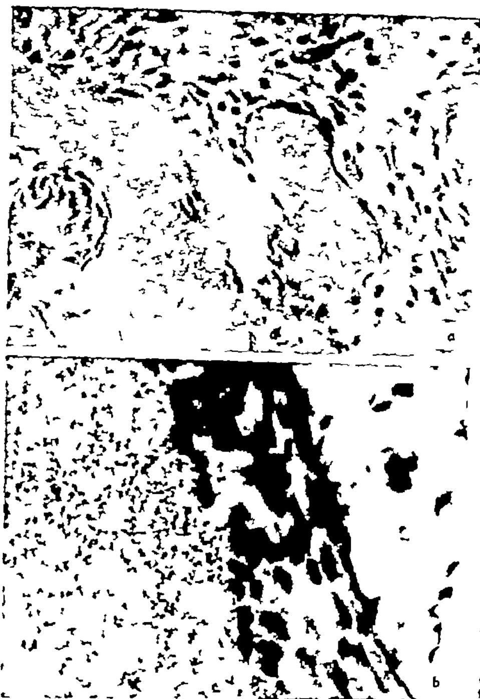


Fig 9 Autoradiographic picture of an ovary of pro-oestrus rat injected with ^{125}I-albumin. (a) Note high activities in the blood-vessels and some activity in the loose connective tissue. ×300. (b) Note high activities in the follicular fluid. ×920.

the liver and the heart can be blocked with lead shields. The concentration of marked gonadotrophins in the partly luteinized ovarian tumour tissue could also be shown by means of autoradiography.

A relatively high concentration of ^{125}I HCG could be seen in normal pro-oestrus females in their luteinized ovaries, confirming observations by Hreshchyshyn & Kazeto (4, 5) in dogs and mice, and in mice by Eshkol et al. (3). ^{125}I-prolactin showed principally the same distribution as ^{125}I HCG but its uptake in ovaries and ovarian tumour tissue seemed lower and of shorter duration. Marked bovine albumin had no specific affinity for those tissues.

It might be possible to obtain similar results in humans. Improved diagnostic methods for ovarian tumours are badly needed. Attempts and trials should then also be made with marked LH and FSH preparations, which might have a higher specific affinity for all or certain types of ovarian tumours. Any ^{125}I-uptake by the thyroid tissue



Fig. 10. Autoradiographic picture of an ovary of pro-oestrus rat injected with ^{125}I-prolactin. The activity is randomly distributed in the corpus luteum. ×920.

should be prevented by premedication with Lugol' solution. The human liver lies at a good distance from the ovaries and can be shielded.

Labelling with other isotopes should also be tested, giving possible improvements in the gamma-camera technique for this type of diagnosis with less loading and better image. The use of isotopes in this respect could also—because of the intracellular enrichment seen at autoradiography—make possible the intracellular radiation treatment of ovarian tumours.

ACKNOWLEDGEMENTS

The NIH-P-B-2 Bovine Prolactin, National Institute of Health, is distributed by Endocrinology Study Section NIAMD, Bethesda, Md.

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Table II. *Activities in several tissues in relation to the activity in the blood found at autopsy of the different experimental animals listed in Table I*

Activity in tissue (cpm/g) / Activity in blood (cpm/g)

Tissue	Case I.1	I.2	I.3	II.1	II.2	II.3	III.1	III.2	IV.1	IV.2	V.1	V.2
Tumour	—	—	—	1.39	1.56	1.14	—	—	—	—	0.43	0.59
Ovaries	—	3.14	12.48	—	—	—	—	0.37	—	0.75	—	—
Liver	0.58	0.64	1.02	0.85	0.61	0.84	0.57	0.28	1.18	1.02	0.66	0.40
Kidney	2.63	2.85	2.02	2.29	3.46	3.19	0.48	0.22	4.63	0.97	1.87	1.90
Spleen	0.65	0.76	0.73	0.66	0.49	0.42	0.39	0.34	0.51	0.54	0.33	0.44
Adrenal	0.34	0.68	0.62	0.53	0.68	0.54	0.58	0.47	1.31	0.61	0.70	0.71
Uterus	0.23	0.23	0.52	0.14	0.12	0.31	0.21	0.08	0.17	7.76	0.18	0.43
Hypophysis	0.30	0.24	0.52	0.19	0.45	0.45	0.54	0.26	0.39	0.27	0.19	0.16
Muscle	0.12	0.09	0.13	0.15	0.11	0.10	0.04	0.04	0.04	0.08	0.06	0.06
Fat	0.09	0.02	0.07	0.07	0.12	0.05	0.03	0.01	0.00	0.03	0.02	0.01

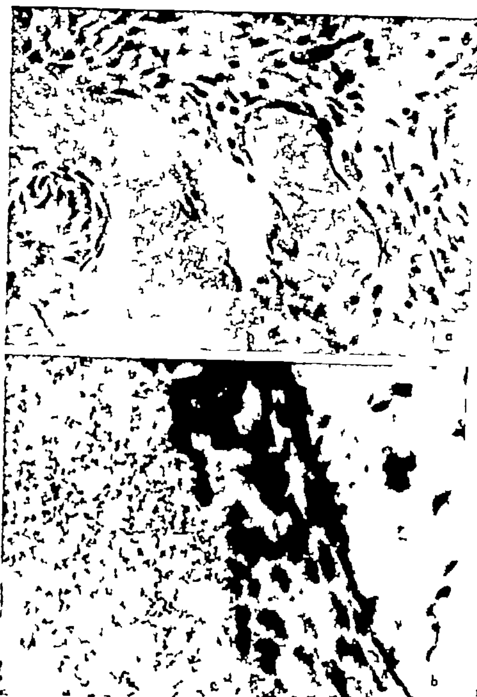


Fig 9 Autoradiographic picture of an ovary f a pro-oestrus rat injected with [131]I-albumin. ( ) Note high activities in the blood-vessels and some activity in the loose connective tissue. 300 (*b*) Note high activities in the follicular fluid. 920.

the liver and the heart can be blocked with lead shields. The concentration of marked gonadotrophins in the partly luteinized ovarian tumour tissue could also be shown by means of autoradiography

A relatively high concentration of [131]I HCG could be seen in normal pro-oestrus females in their luteinized ovaries confirming observations by Hrushchyshyn & Kazeto (4 5) in dogs and mice, and in mice by Eshkol et al. (3) [131]I prolactin showed principally the same distribution as [131]I HCG but its uptake in ovaries and ovarian tumour tissue seemed lower and of shorter duration. Marked bovine albumin had no specific affinity for those tissues.

It might be possible to obtain similar results in humans. Improved diagnostic methods for ovarian tumours are badly needed. Attempts and trials should then also be made with marked LH and FSH preparations, which might have a higher specific affinity for all or certain types of ovarian tumours. Any [131]I uptake by the thyroid tissue



Fig. 10. Autoradiographic picture of an ovary of pro-oestrus rat injected with ¹³¹I-prolactin. The activity is randomly distributed in the corpus luteum. ×920.

should be prevented by premedication with Lugol's solution. The human liver lies at a good distance from the ovaries and can be shielded.

Labelling with other isotopes should also be tested, giving possible improvements in the gamma-camera technique for this type of diagnosis with less loading and better image. The use of isotopes in this respect could also—because of the intracellular enrichment seen at autoradiography—make possible the intracellular radiation treatment of ovarian tumours.

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Table II. *Activities in several tissues in relation to the activity in the blood found at autopsy of the different experimental animals listed in Table I*

Activity in tissue (cpm/g) / Activity in blood (cpm/g)

Tissue	Case I 1	I 2	I 3	II 1	II 2	II 3	III 1	III 2	IV 1	IV 2	V 1	V 2
Tumour	—	—	—	1.39	1.56	1.14	—	—	—	—	0.43	0.59
Ovaries	—	3.14	12.48	—	—	—	—	0.37	—	0.75	—	—
Liver	0.58	0.54	1.02	0.85	0.61	0.56	0.37	0.28	1.18	1.82	0.66	0.40
Kidney	2.63	3.85	2.82	2.29	3.46	3.79	0.48	0.22	4.63	0.97	1.87	1.50
Spleen	0.63	0.76	0.73	0.66	0.49	0.42	0.39	0.34	0.51	0.54	0.33	0.44
Adrenal	0.34	0.68	0.62	0.53	0.68	0.54	0.58	0.47	1.31	0.61	0.70	0.71
Uterus	0.21	0.25	0.52	0.14	0.12	0.31	0.21	0.08	0.17	7.76	0.18	0.43
Hypophysis	0.30	0.24	0.52	0.19	0.45	0.43	0.56	0.26	0.39	0.27	0.19	0.16
Muscle	0.12	0.09	0.13	0.15	0.11	0.10	0.04	0.01	0.04	0.08	0.08	0.06
Fat	0.09	0.02	0.07	0.07	0.12	0.05	0.03	0.01	0.00	0.03	0.02	0.01



Fig 11 Histological picture of an experimental rat ovarian tumour H.E. 300

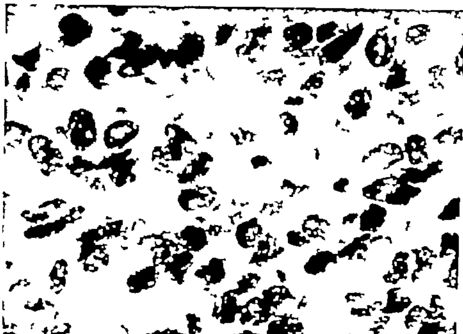


Fig 12 Autoradiographic picture of an experimental ovarian tumour of rat injected with ^{131}I-HCG The activity is randomly distributed in the tumour-tissue. 920.

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ENCIRCLING SUTURE OF THE CERVIX IN PLACENTA PRAEVIA

Ten Years Experience

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Abstract. Over a ten-year period, placenta praevia occurred in 41 instances among 12 485 deliveries (ratio 1 305). In 19 of these, cervical suture was undertaken in order to prevent severe bleeding while prolonging pregnancy. Infants weighing 2 500 g or less were delivered in seven of the 41 cases (17.1%). Of 43 infants, two, of which one was a twin, failed to survive (4.7%). Calculated in terms of single pregnancies, the figures correct to 1.8 and 2.6%, respectively. These figures are lower than any previously published.

The perinatal mortality in placenta praevia remains substantial. This is chiefly due to the high incidence of associated prematurity and presumably to superadded stresses resulting from bleeding. The incidence of prematurity associated with placenta praevia, as quoted in 17 detailed reports, varies from 24 to 54% with a mean value of 39% (Table I). Noack (22) wrote as follows: "The greatest attention must be focussed on the avoidance of prematurity in placenta praevia. Of 80 infants who failed to survive 85% weighed less than 2 500 g. The perinatal mortality in placenta praevia has diminished in recent years. The ten lowest figures reported vary between 8 and 12.6% —the average being 10.8 (Table II). Treatment, hitherto strictly conservative, has been founded on bed rest and, to a very great extent, on Caesarean section.

METHOD

In 1959 the Norwegian obstetrician Lövset (15) described a method which he had been using for ten years and which he claimed was extremely effective, not only in stopping massive haemorrhage, but also in promoting the continuation of pregnancy towards full term. Lövset published no figures however.

The method consists of a simple circumferential suture or encircling of the cervix, which is usually applied as a purse-string suture encompassing the cervix high up in the fornices and taking shallow bites into the cervical tissue. This technique was also described by McDonald (17). It is convenient to tie the knots in the anterior fornix, leaving the ends long to facilitate subsequent removal. In cases of severe haemorrhage at full term, two tenacula can be applied side by side through the entire cervix, to serve the same purpose. This manoeuvre can also be used with advantage when performing encircling.

General anaesthesia is to be preferred.

The present writer began to use Lövset's method in 1960 and the results from a ten-year period can now be studied.

Some of the details have previously been presented (von Friesen (8)) in an article describing fifty seven patients treated in this way. No other results have thus far been forthcoming, except for an account of two cases published by Ardillo (1). Rawlings (25) has, in fact, employed "ligature of the cervix" prophylactically or therapeutically in cases of recurrent abortion or premature labour. Among 100 such cases, there were four in whom placenta praevia was subsequently diagnosed. These pregnancies came to an end at the 40th, 36th, 38th and 22nd weeks, respectively. Rawlings' narr I was undecided that ligation would be valueless in early repeated bleeding *except, perhaps, in placenta praevia.*

RESULTS

During the period 1960–1969 there were 41 confined cases of placenta praevia among the 12 485 deliveries at the Obstetrical Department at Lidköping. This constitutes one out of every 305 deliveries, or 0.33%—a rather low figure. There were two instances of twin births and thus in all 43 infants were born.

Suture of the cervix was performed in 19 of these 41 cases—generally early cases. In patients

Table I *Placenta praevia. Prematurity*

	%	No. of cases
Williams, 1948	30	105
Beilly et al., 1952	33	91
Westgren, 1954	36	350
Grant, 1955	24	200
Noack, 1958	42	1 466
Semmens, 1959	40	166
Smith, 1959	45	99
Green, 1959	27	242
Lepage et al., 1963	38	114
Menon, 1963	47	410
Foscolos, 1964	45	495
Nieminen et al., 1964	35	420
Pedowitz, 1965	37	226
Hibbard 1969	52	475
de Villiers, 1969	39	90
Castelazo-Ayala et al., 1970	54	1 000
Pallier et al., 1970	36	140
Mean	39 ± 0.64 (1) Total	6 089
von Friesen, 1971	17 1 ± 5.9 (2)	41
	Difference (1)–(2) = 22 ± 5.9	

approaching term, immediate delivery was generally undertaken Acute severe haemorrhage was effectively dealt with by encircling suture in two patients. The suture was allowed to remain in place for more than nine weeks in five of our patients (in one instance for 21 weeks!) from five to nine weeks in six patients and from two to four weeks in three patients. On five occasions delivery had to be undertaken within two weeks because of bleeding or pains. Seven patients returned to hospital because of bleeding after more than two weeks and had to be delivered earlier than had been anticipated. In yet others, minor bleeding

Table II. *Placenta praevia. Perinatal mortality*

	%	No. of cases
Beilly et al., 1952	9 8	91
Grant, 1955	11.9	200
Barry et al., 1958	8.0	80
Green, 1959	12.6	242
Kimbrough, 1959	11 1	169
Foot et al. 1960	12.5	120
Macafee, 1960	11.9	200
Foscolos, 1964	8.1	495
Morgan, 1965	9 6	538
Pedowitz, 1965	12.4	226
Mean	10.8 ± 0.64 (1) Tot.	2 361
von Friesen, 1971	4 7 ± 3.2 (2)	43
Difference (1)–(2) =	6.1 ± 3.3	

Table III. *Method of delivery in placenta praevia*

	Treated by encirclage	Treated otherwise
Artificial rupture of membranes and unassisted delivery	2*	3*
Vacuum extraction	2*	3
Caesarean section	16	17
	20	23

* One twin.

and pains dictated that the pregnancies could not be permitted to continue. In five cases, prolongation of pregnancy to within 14 days of the expected date of delivery was achieved. Methods of delivery are summarized in Table III.

Confirmation of the diagnosis was obtained by vaginal examination, by soft tissue X-ray by pelvic angiography or at Caesarean section (Table IV) It will be noted that in most of those not treated by encirclage the diagnosis was confirmed by vaginal examination or at Caesarean section.

Prematurity

In 7 of our 41 cases (17 1 %) the infants weighed 2 500 g or less. If multiple pregnancies are excluded the incidence of prematurity falls to 12.8 % —These are low figures compared with those quoted elsewhere.

Figures from authors who have published detailed accounts are summarized in Table I. The average value for prematurity here is 39 ± 0.64 % The difference, when compared with our figures, is 22 ± 5 9 % and thus statistically significant.

Perinatal mortality

This term includes stillbirths where death occurred after the 28th week of pregnancy smaller

Table IV *Confirmation of diagnosis in placenta praevia*

	Treated by encirclage	Treated otherwise
Soft-tissue X-ray	6	5
Pelvic arteriography	11	2
Vaginal examination or at Caesarean section	2	15
	19	22

fetuses who had breathed or shown other signs of life, and infants succumbing within seven days of birth.

The present series includes 43 infants of whom 2 (4.7 ± 3.2 %) failed to survive. Both were premature. Exclusion of multiple births reduces this figure to 2.6 %. The ten best results reported by other authors are summarized in Table II. The perinatal mortality here averages 10.8 ± 0.64 %. The difference as compared with our own results is not statistically significant (6.1 ± 3.5 %). The figures quoted by most other investigators are considerably higher, however, and since prematurity—the main cause of perinatal mortality in placenta praevia—is considerably less common among our patients, it is reasonable to assume that the difference would be significantly lower in a more extensive study.

These results were obtained despite an initially somewhat permissive attitude to those who had been treated in this way. No less than 15 of 19 patients were discharged from hospital only a few days after "encirclage" and some were even allowed to resume light work. Latterly, however, we ha prescribed complete rest at home and prophylactic Isoxsuprine (Duvadilan®) 10 mg orally three times a day—in the hope of preventing the onset of premature labour—with the recommendation that an e tra dose be taken in the event of bleeding or the onset of pain. W ha e also considered the ad isability of giving tranexamic acid (Cyklokapron®) in the event of bleeding to reduce the bleeding tendency.

The tw infants failing to surviv were both premature.

Case 1

Twin pregnancy. Suture of the cervix performed at 21st week. Membranes ruptured spontaneously at 28th week—patient became febrile one week later. Liquor normal. Fetal prognosis deemed poor. X-ray examination as not undertaken. The circumferential suture was removed and nasal Oxytocin (Partocon®) given. After uneventful delivery of the healthy first twin the patient began to bleed heavily. The placenta was clearly felt through the cervix. Rapid extraction of the second twin became necessary. This infant, hich weighed 1 450 g, expired shortly after birth.

Case 2

Suture of the cervix performed during the 33rd eek because of heavy bleeding. Further bleeding occurred 2 week later. X-ray findings suggested lateral placenta praevia. The circumferential suture was removed and the membranes artificially ruptured. Delivery during which the child succumbed could only be achieved by means of difficult vacuum-extraction. The child weighed 2 320 g. The placenta was in fact found to be located very low. *Retrospectively* it must be concluded that this case should have been managed differently and correct diagnosis would hav led, in the normal course of events, to Caesarean section.

DISCUSSION

Nesbitt (20) wrote: "The mode of production of haemorrhage in placenta praevia is somewhat controversial, although the best explanation would seem to be that the progressive formation of the lower uterine segment and dilatation of the internal cervical os result in torn placental attachments and rupture of marginal veins at the periphery of the placenta or maternal sinuses in the decidual plate.

Our results support Nesbitt's views, since the effect of encircling suture of the cervix is probably to fix and support the lower uterine segment.

The earlier the bleeding occurs the slighter it tends to be. Early diagnosis is important. We have confirmed the diagnosis of placenta praevia by pelvic angiography as described by Fernström (5) as early as the 24th to 25th week of pregnancy but we hav also obtained soft-tissue X-ray pictures demonstrating normal placental location at the 27th week.

To summarize we can claim to have succeeded in reducing the prematurity rate and hence the perinatal mortality by the use of cervical suture in a small series of patients with placenta praevia.

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TOTAL PROTEIN CONTENT IN AMNIOTIC FLUID FROM NORMAL PREGNANCIES AND FROM PREGNANCIES COMPLICATED BY RH ISOIMMUNIZATION

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Abstract. Amniotic fluid total protein content was measured in 129 samples from 112 normal pregnancies and 223 samples from 118 pregnancies complicated by Rh-isoimmunization. A few samples were also obtained from pregnancies with other complications. A biuret method was used. The results showed that the total protein content is elevated in cases of mild and of very serious fetal disease. Intermediate stages of isoimmunization were associated with comparatively low protein levels. This may indicate that a high protein level may have a protective influence against the effects of isoimmunization. Because of the inability to discriminate between mild and very serious cases of isoimmunization protein estimations will be of limited value in the practical management of isoimmunized pregnancies. Due to the progressive decrease in total protein concentrations after the 24th week in normal pregnancies protein estimations may be of some value in determining fetal age.

The protein content of amniotic fluid has been investigated by several authors, both in normal pregnancies (1, 6, 8, 15, 18, 24) and in pregnancies complicated by Rh-isoimmunization (5, 14, 15, 22, 23). However, the levels given are not always related to the duration of pregnancy and the series are sometimes rather small. Opinions still differ concerning the relative importance of alterations in the protein content of the amniotic fluid in connection with Rh-isoimmunization.

In the present investigation the total protein content of the amniotic fluid has been measured during a large number of normal pregnancies and during pregnancies complicated mainly by Rh-isoimmunization. The results have been related to gestational age and the levels from complicated pregnancies related to the levels from the normal pregnancies. This investigation is part of a larger study of factors in amniotic fluid that may be of importance in evaluating fetal health.

MATERIAL

Normal. Samples were obtained from two groups of women. Fifty-five samples of amniotic fluid were obtained from women with pregnancies of 13 to 24 weeks duration, who were admitted for therapeutic abortions. In late pregnancy 74 samples were collected from 57 women. Most of the latter samples came from patients with previous Rh-isoimmunization but in whom the baby from the current pregnancy was Rh negative and healthy. Some samples were obtained at the time of elective Caesarean section in patients with narrow pelvis. All these pregnancies were otherwise normal as far as could be judged from ordinary clinical criteria.

From complicated pregnancies 223 amniotic fluid samples were obtained in 118 pregnancies associated with Rh-isoimmunization and affected foetuses. All the children from these pregnancies were Rh positive and had positive direct Coombs' test except those who died in utero and did not have their blood grouped. The Rh-isoimmunized pregnancies were classified according to the status of the infants. Pregnancies with mildly affected infants requiring at most one exchange transfusion and with a cord blood haemoglobin of 12.1 g/ml or higher were classified as group I. Group II included pregnancies which resulted in moderately affected infants with a cord blood haemoglobin of 8.1–12.0 g/100 ml or requiring two to three exchange transfusions. Group III contained infants with a cord blood haemoglobin of 8.0 g/100 ml or lower who required more than three exchange transfusions. Group IV are pregnancies which resulted in foetal perinatal death due to erythroblastosis. 12 samples were obtained from 12 patients with the diagnosis hepatosis gravidarum. These patients were not isoimmunized and the pregnancies were otherwise normal. Samples were also obtained from two patients with preeclamptic toxaemia and three patients with polyhydramnios.

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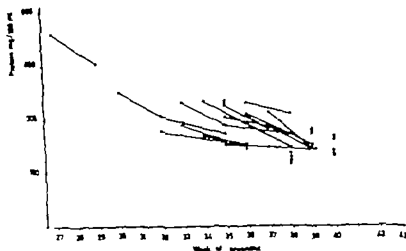


Fig. 2. Individual values from the later part of normal pregnancies. Lines connect consecutive values from the same patient.

alues from the later part of normal pregnancies are given in Fig. 2.

For normal pregnancies terminated within 7 days of sampling (38th–40th week) no correlation was found between total protein values and fetal birthweight (= 29) placental weight ($n = 28$) or infant cord blood haemoglobin ($n = 31$).

Rh-isoimmunized pregnancies group I showed a significant elevation of the mean levels for the 33rd–34th and 35th–36th week compared with the normals ($0.02 > p > 0.01$) (Table I). The levels for the 37th–38th week and 39th–40th week were not significantly different from the normal means. Rh-isoimmunized pregnancies group II showed no significant difference against the normal means for the 33rd–34th, 35th–36th and 37th–38th week but significantly lower mean values compared with the group I pregnancies for the 33rd–34th week ($0.02 > p > 0.01$) 35th–36th week ($0.005 > p > 0.001$) and 37th–38th week ($0.05 > p > 0.025$) (Table I). For iso-immunized pregnancies group III (Fig. 3 and Table I) there was no significant difference against the normals for the 33rd–34th, 35th–36th and 37th–38th week. Nor was there

Table I. *Mean total protein levels in amniotic fluid. Normal and Rh-isoimmunized pregnancies*

Mg/100 ml ± S.E.M. Figures in brackets indicate number of samples

Diagnosis	Week of pregnancy 25–26	27–28	29–30	31–32	33–34	35–36	37–38	39–40	40–41
Normal pregnancies	—	340 (1)	415.5 (2)	285.0 (2)	291.6 ±22.1 (5)	270.6 ±13.0 (16)	231.4 ±13.6 (18)	225.8 ±9.4 (24)	162.0 ±13.4 (5)
Rh Group I	—	—	—	828 (1)	486.0 ±69.5 (3)	347.2 ±28.6 (13)	275.4 ±12.5 (19)	248.4 ±22.6 (15)	—
Rh Group II	—	—	—	432.0 ±39.0 (4)	331.9 ±18.3 (17)	267.0 ±9.9 (22)	244.4 ±7.9 (26)	211.5 ±13.9 (6)	—
Rh Group III	—	593.8 ±34.1 (4)	604.3 ±39.9 (9)	449.5 ±40.5 (6)	353.8 ±22.0 (15)	261.8 ±12.2 (19)	244.7 ±20.9 (14)	—	—
Rh Group IV	693.3 ±137.4 (3)	—	528.0 (2)	483.2 ±19.3 (6)	427.2 ±26.7 (5)	394.4 ±29.1 (7)	334.5 ±40.1 (4)	304 (1)	—

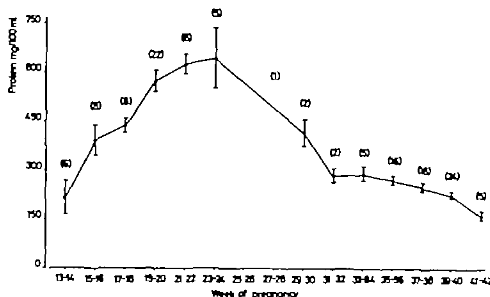


Fig. 1. Normal pregnancies. Mean values ±S.E.M. Figures within brackets indicate number of samples.

All samples were taken by abdominal amniocentesis as previously described (9) or by puncture through the uterine wall during Caesarean section. None of the patients was in labour. Macroscopically bloodstained samples were discarded, as were samples from patients with uncertain menstrual data. After amniocentesis the samples were immediately centrifuged and filtered through fine filter paper with addition of a small amount of inert filter powder (Supercel) to remove all suspended particles. The samples were then kept in a refrigerator until analysis, which was usually done within a few days.

METHODS

Estimations of the total protein content were made according to the biuret method as described by Burgi et al. (3). This method includes the use of both a sample blank and a reagent blank. The biuret reaction is considered to be specific for compounds with at least two peptide bonds (10). It is thus not specific for proteins but gives a colour reaction also with peptides and the amino acid histidine. With these reservations the reaction is generally accepted as a reliable method for protein estimations.

The sample volume necessary for one duplicate estimation was *1.0 ml. The extinction values were read on a* Beckman B spectrophotometer. The net extinctions were transformed to mg protein per 100 ml with the help of a standard curve obtained from known dilutions of pure bovine albumin (Armour). The standard curve was straight for 40 to 400 mg/100 ml which was the range used for measuring. To avoid too high extinction values the samples were usually diluted 1:3 with isotonic saline. All reagents used were of analytical grade. The majority of samples were estimated in duplicate. The coefficient of variation was 4.2% computed from consecutive duplicate estimations ($n=72$) and it was found to be 4.0% for repeated estimations of samples kept in a refrigerator for up to one month ($n=72$). Storing of samples for at least one month thus did not seem to affect the protein content. To test if nitrogen compounds normally present in amniotic fluid would affect the determinations urea, uric acid and creatinine were added to samples of amniotic fluid in concentrations of 30, 10 and 2 mg/100 ml respectively. Addition of urea decreased the values 1.5 mg/100 ml, uric acid increased the values 3.0 mg/100 ml and all three compounds together increased the readings 0.5 mg/100 ml ($n=6$). These deviations are not significant.

RESULTS

The mean levels were calculated for 2-week periods. In normal pregnancies the amniotic fluid protein increased from 214.5 mg/100 ml in the 13th–14th week to a peak of 643.2 mg/100 ml in the 23rd–24th week (Fig. 1). From then on there was a gradual decline throughout the remaining course of pregnancy. The increase from the 13th–14th week to the 15th–16th week (214.5–391.5 mg/100 ml) was probably significant ($0.05 > p > 0.025$) as was also the rise from the 17th–18th week to the 19th–20th week (437.3–573.1 mg/100 ml), ($0.025 > p > 0.020$). The difference between the mean level for the 19th–20th week and the mean levels for the 21st–22nd week (625.5 mg/100 ml) and the 23rd–24th week was not significant. The peak level at 23–24 weeks was significantly different from the mean level at 33–34 weeks ($0.01 > p > 0.005$) (Table I). The decline from the mean level at 35–36 weeks to the mean level at 39–40 weeks was significant ($0.01 > p > 0.005$) but not the difference between the 37th–38th week and the 39th–40th week. The mean levels for the 39th–40th week and the 41st–42nd week were significantly different ($0.01 > p > 0.005$). Individual

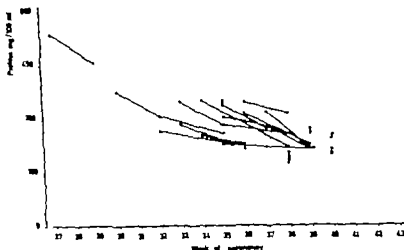


Fig. 2. Individual values from the later part of normal pregnancies. Lines connect consecutive values from the same patient.

values from the later part of normal pregnancies are given in Fig. 2.

For normal pregnancies terminated within 7 days of sampling (38th–40th week) no correlation was found between total protein values and fetal birthweight ($n = 29$), placental weight ($n = 28$) or infant cord blood haemoglobin ($n = 31$).

Rh-isoimmunized pregnancies group I showed a significant elevation of the mean levels for the 33rd–34th and 35th–36th week compared with the normals ($0.02 > p > 0.01$) (Table I). The levels for the 37th–38th week and 39th–40th week were not significantly different from the normal means. Rh-isoimmunized pregnancies group II showed no significant difference against the normal means for the 33rd–34th, 35th–36th and 37th–38th week, but significantly lower mean values compared with the group I pregnancies for the 33rd–34th week ($0.02 > p > 0.01$), 35th–36th week ($0.005 > p > 0.001$) and 37th–38th week ($0.05 > p > 0.025$) (Table I). For iso-immunized pregnancies group III (Fig. 3 and Table I) there was no significant difference against the normals for the 33rd–34th, 35th–36th and 37th–38th week. Nor was there

Table I. *Mean total protein levels in amniotic fluid. Normal and Rh-isoimmunized pregnancies*

Mg/100 ml ± S.E.M. Figures in brackets indicate number of samples

Diagnosis	Weeks of pregnancy 25–26	27–28	29–30	31–32	33–34	35–36	37–38	39–40	40–41
Normal pregnancies	—	540 (1)	415.5 (2)	285.0 (2)	291.6 ±22.1 (5)	270.6 ±13.0 (16)	251.4 ±13.6 (18)	225.8 ± 9.4 (24)	162.0 ±13.4 (5)
Rh– Group I	—	—	—	628 (1)	486.0 ±69.5 (3)	347.2 ±28.6 (18)	275.4 ±12.5 (19)	248.4 ±22.6 (15)	—
Rh– Group II	—	—	—	432.0 ±39.0 (4)	331.9 ±18.3 (17)	267.0 ± 9.9 (22)	244.4 ± 7.9 (26)	211.5 ±13.9 (6)	—
Rh Group III	—	597.8 ±36.1 (4)	604.3 ±39.9 (9)	449.5 ±40.5 (6)	358.8 ±22.0 (15)	261.8 ±12.2 (19)	244.7 ±20.9 (14)	—	—
Rh Group IV	693.3 ±137.4 (3)	—	328.0 (2)	483.2 ±19.3 (9)	427.2 ±26.7 (5)	394.4 ±29.1 (7)	334.5 ±40.1 (4)	306 (1)	—

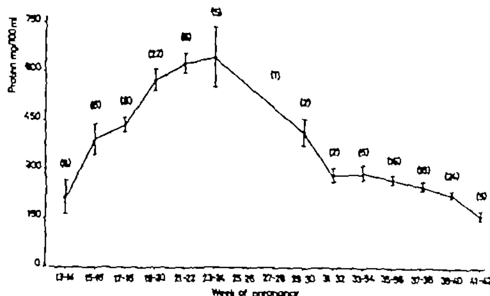


Fig. 1. Normal pregnancies. Mean values ±S.E.M. Figures within brackets indicate number of samples.

All samples were taken by abdominal amniocentesis as previously described (9) or by puncture through the uterine wall during Caesarean section. None of the patients was in labour. Macroscopically bloodstained samples were discarded, as were samples from patients with uncertain menstrual data. After amniocentesis the samples were immediately centrifuged and filtered through fine filter paper with addition of a small amount of inert filter powder (Supercel) to remove all suspended particles. The samples were then kept in a refrigerator until analysis, which was usually done within a few days.

METHODS

Estimations of the total protein content were made according to the biuret method as described by Bürgi et al. (3). This method includes the use of both a sample blank and a reagent blank. The biuret reaction is considered to be specific for compounds with at least two peptide bonds (10). It is thus not specific for proteins but gives a colour reaction also with peptides and the amino acid histidine. With these reservations the reaction is generally accepted as a reliable method for protein estimations.

The sample volume necessary for one duplicate estimation was 1.0 ml. The extinction values were read on a Beckman B spectrophotometer. The net extinctions were transformed to mg protein per 100 ml with the help of a standard curve obtained from known dilutions of pure bovine albumin (Armour). The standard curve was straight for 40 to 400 mg/100 ml which was the range used for measuring. To avoid too high extinction values the samples were usually diluted 1:3 with isotonic saline. All reagents used were of analytical grade. The majority of samples were estimated in duplicate. The coefficient of variation was 4.2% computed from consecutive duplicate estimations ($n = 72$) and it was found to be 4.0% for repeated estimations of samples kept in refrigerator for up to one month ($n = 72$). Storing of samples for at least one month thus did not seem to affect the protein content. To test if nitrogen compounds normally present in amniotic fluid would affect the determinations urea, uric acid and creatinine were added to samples of amniotic fluid in concentrations of 30, 10 and 2 mg/100 ml respectively. Addition of urea decreased the values 1.5 mg/100 ml, uric acid increased the values 3.0 mg/100 ml and all three compounds together increased the readings 0.5 mg/100 ml ($n = 6$). These deviations are not significant.

RESULTS

The mean levels were calculated for 2 week periods. In normal pregnancies the amniotic fluid protein increased from 214.5 mg/100 ml in the 13th–14th week to a peak of 643.2 mg/100 ml in the 23rd–24th week (Fig. 1). From then on there was a gradual decline throughout the remaining course of pregnancy. The increase from the 13th–14th week to the 15th–16th week (214.5–391.5 mg/100 ml) was probably significant ($0.05 > p > 0.025$) as was also the rise from the 17th–18th week to the 19th–20th week (437.3–573.1 mg/100 ml) ($0.025 > p > 0.020$). The difference between the mean level for the 19th–20th week and the mean levels for the 21st–22nd week (625.5 mg/100 ml) and the 23rd–24th week was not significant. The peak level at 23–24 weeks was significantly different from the mean level at 33–34 weeks ($0.01 > p > 0.005$) (Table I). The decline from the mean level at 35–36 weeks to the mean level at 39–40 weeks was significant ($0.01 > p > 0.005$) but not the difference between the 37th–38th week and the 39th–40th week. The mean levels for the 39th–40th week and the 41st–42nd week were significantly different ($0.01 > p > 0.005$). Individual

Table II. *Total protein in amniotic fluid compared with cord blood haemoglobin. Rh-isoimmunized pregnancies*

Mg/100 ml. Means ± S.E.M. denotes significant difference compared with the normal levels

Weeks of pregnancy	Cord blood haemoglobin g/100 ml					
	>14.1	14.0–12.1	12.0–10.1	10.0–8.1	8.0–6.1	<6.0
27–28	—	—	—	=2 580.0	1 498	—
29–30	—	—	—	5 595.8 ±54.2	=2 684.0	=1 516
31–32	—	2 430.5	=2 340.0	6 442.5 ±31.1	=1 480	3 491.3 ±39.8
33–34	5 406.8 ±62.2	n=5 353.2 ±36.0	=5 322.2 ±38.1	=11 324.5 ±19.9	=7 407.1 ±30.0	=3 764.7 ±281.1
35–36	13 346.1 ±29.4	16 281.2 ±19.7	=9 314.1 ±27.6	6 233.0 ±15.7	=5 253.6 ±22.1	=5 341.4 ±20.7
37–38	20 266.7 ±11.4	13 258.9 ±16.4	=11 235.8 ±10.9	=4 206.8 ±19.3	=3 234.0 ±31.2	=4 339.0 ±52.5
39–40	8 273.1 ±28.2	6 239.7 ±35.6	2 208.0			

...nez et al. (19, 20) slightly higher values have been reported by Queenan et al. (15), Crosignani & Polvani (6) and slightly lower values by Watson et al. (23) and Wild (24). Comparison with previous reports is sometimes difficult as several investigators have not stated the exact stage of pregnancy when samples were taken. As may be seen from this study there are pronounced variations in amniotic fluid protein concentration as pregnancy advances. This is in accord with the findings of Queenan et al. (15). The reasons for these variations are still obscure. Current opinion favours the concept that the maternal serum is the main source of amniotic fluid proteins (1, 7, 16, 18, 19, 20, 21) but some have also found similarities with cord blood serum (13, 24). The present study does not support either view as it consists only of quantitative estimations. The rise in protein concentration during early pregnancy may be due to alterations in the fetal membranes facilitating penetration of proteins or to accumulation of protein. The successive decline in protein concentration after the 24th–25th week may be due to increasing fetal deglutition which removes fluid and proteins in bulk. Abbas & Tovey (1) have pointed out the possibility that amniotic fluid may be of importance for fetal nutrition. In the present series there was one case of polyhydramnios with fetal oesophageal atresia and

Table III. *Total protein levels in amniotic fluid. Pregnancies with other complications*

Mean ± S.E.M. Mg/100 ml. Figures within brackets indicate number of samples

Diagnosis	Weeks of pregnancy						
	29–30	31–32	33–34	35–36	37–38	39–40	41–42
Hepatosis gravidarum	414 (1)	—	315 (1)	240 (1)	279.0 ±49.5 (6)	258.0 (2)	312 (1)
Preeclamptic toxaemia	—	—	—	300 (1)	—	189 (1)	—
Hydramnios without malformations	232 (1)	—	—	—	—	135 (1)	—
Hydramnios a. oesophageal atresia	—	—	—	—	714 (1)	—	—

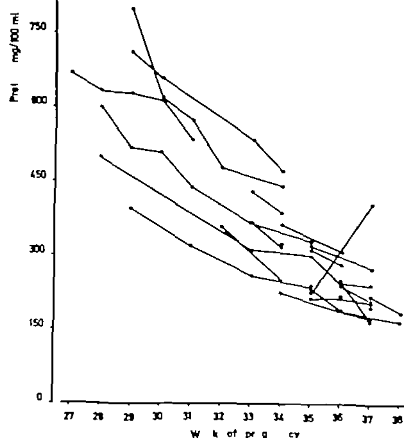


Fig 3 Individual values from Rh-isoimmunized pregnancies group III. Lines connect consecutive values from the same patient.

any significant difference against group II pregnancies for comparable periods. The mean values for the 33rd–34th week and the 35th–36th week were significantly lower than the group I values ($0.05 > p > 0.025$ and $0.025 > p > 0.01$) but the difference for the 37th–38th week was not significant. The Rh pregnancies belonging to group IV showed significantly elevated mean values compared with the normal pregnancies for the 33rd–34th, 35th–36th and 37th–38th week ($0.005 > p > 0.001$, $0.001 > p$ and $0.025 > p > 0.020$ respectively). The difference against the group I pregnancies was not significant for the 35th–36th and 37th–38th week. Compared with group II there were significantly elevated mean levels for the 33rd–34th, 35th–36th and 37th–38th week ($0.02 > p > 0.01$, $0.001 > p$ and $0.005 > p > 0.001$ respectively). The difference between group III and group IV pregnancies was significant only for the 35th–36th week ($0.001 > p$). In calculating the mean values for group IV pregnancies three extremely high values have been eliminated, they belonged to samples obtained shortly before intrauterine foetal death (4 300 in the 26th week, 1 860 in the 31st week and 1 325 mg/100 ml in the 33rd week).

A comparison was also made between the total protein mean levels and the cord blood haemoglobin values for the isoimmunized pregnancies (Table II). Significantly elevated levels compared with the normal means occurred in the highest and the lowest haemoglobin classes. The intermediate haemoglobin classes showed a tendency to low values.

Pregnancies complicated with hepatosis gravidarum showed no significant difference compared with the normals for the 37th–38th week (Table III). The two patients with preeclamptic toxaemia showed values within the normal range. Two patients with polyhydramnios without foetal malformations showed comparatively low values, but one patient with polyhydramnios and fetal oesophageal atresia had a very high value (Table III).

DISCUSSION

The total amniotic fluid protein values obtained for early normal pregnancies correspond well with those reported by others (15, 17). For late pregnancies there is good correlation with the values reported by Abbas & Tovey (1), Fischbacher & Quinlivan (8), Stander et al. (18), Usuategui-Go-

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this fluid had a high protein content, indicating that fetal deglutition may be of importance in removing protein.

Previous studies have indicated elevated protein levels in cases of severe Rh isoimmunization (5 14 15 22, 24) The present study indicates that the picture is more complicated. Two groups of Rh-isoimmunized pregnancies showed definitely elevated levels, firstly the most seriously affected cases with perinatal fetal death from erythroblastosis, secondly the mildly affected cases. Pregnancies which resulted in surviving babies with moderate to severe Rh disease had relatively low protein levels compared with the normals. The grouping of the Rh cases used here corresponds largely with that used by others (5 11 12, 15) The elevated levels found in cases of mild Rh disease are difficult to explain. In pregnancies complicated by Rh disease there seems to exist an association between protein and bilirubin content of the amniotic fluid (5 24) In normal pregnancies the bilirubin content of the amniotic fluid shows a distribution at different stages of pregnancy which is very similar to that found here for protein (2 4) This is not surprising as bilirubin in amniotic fluid is bound to proteins, mostly albumin (23) It might be that a high protein content by binding bilirubin gives some protection against the effects of Rh isoimmunization. The difference between the mildly affected cases and the other Rh groups seems to be less pronounced during the last weeks of pregnancy Due to the lack of samples from normal pregnancies during the interval 25th to 32nd week it has only been possible to evaluate the difference after that period but the few values available indicate that the differences exist also during that part of pregnancy It might be that the variations in protein content of the amniotic fluid reflect differences in penetration of substances into the amniotic cavity rather than being a result of the Rh disease as such. The elevated levels found in Rh group IV may be due to anoxic tissue damage caused by fetal anaemia. The highest protein levels were found in association with a hydropic fetus.

The grouping of the Rh cases may be somewhat subjective as it is influenced by the treatment given to the infants. This was the reason for comparing the amniotic fluid protein content with the cord blood haemoglobin Cord blood haemoglobin values are considered to be the best single indicator of infant status in Rh-isoimmunized pregnancies (11 12) It was found that high protein levels occur in connection with high and with very low haemoglobin concentrations but that the intermediate haemoglobin levels do not differ from the normal means. In normal pregnancies there could not be found any association between amniotic fluid protein concentrations and cord blood haemoglobin values.

Hepatosis gravidarum did not seem to have any influence on amniotic fluid protein levels. Four of the samples in this group had meconium stained fluids but the protein values fell within the normal range.

The two samples from pregnancies with poly hydramnios without malformations showed low protein values which may reflect an effect of dilution.

From the results of this study it is clear that estimations of total protein concentration in amniotic fluid would be of limited practical value in cases of Rh-immunization. It is not possible to discriminate between mild and very serious cases with any certainty As part of a test battery to evaluate fetal maturity in otherwise normal pregnancies protein determinations may be of some value as a total protein concentration below 180 mg/100 ml usually means that the pregnancy has reached at least the 38th week.

ACKNOWLEDGEMENTS

The autho is indebted to Dr Elof Johansson and the personnel of the Primate Laboratory for Reproductn Research at Uppsala for kind help with the manuscript. This study was supported by the University f Uppsala

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In Memoriam



At our last Scandinavian Congress in Turku we knew that *Finn Bøe* was suffering from recurrence of an oral cancer. This did not however interfere with his pattern of life. He continued his daily routine and scientific work until death suddenly struck him on October 28th, 1970 and mercifully spared him further sufferings.

Dr med Finn Bøe was born May 30th 1906 in Bergen and graduated in 1933 from Oslo University. Soon after his graduation he started his clinical training in obstetrics and gynecology in Bergen. Two years later his everlasting inclination for research brought him to the Pharmacological Institute of Oslo University where he stayed 2 years and got his basic training in scientific methods. He returned to clinical work in departments of obstetrics and gynecology, first three years in the Oslo Municipal Hospital under Dr Kr Skajaa and then 6 years in Oslo University Hospital under professor A. Sunde. Realizing the importance of surgical technique for our speciality he afterwards took one year of training under professor C. Semb in his surgical department in Oslo Municipal Hospital. From 1948 to 1953 he practiced as a specialist in obstetrics and gynecology in Oslo and at the same time he was actively engaged in the first Norwegian laboratory for hormonal assays at the pharmaceutical factory of Nyegaard & Co.

In 1953 he was appointed consultant and in 1955 head of the newly established department of obstetrics and gynecology at Aker Hospital, part of the Oslo Municipal Hospital organization. He stayed there until his death. In 1960 he took a year off to serve as head of the department of obstetrics and gynecology at the National Medical Center Seoul, Korea.

He was on the editorial board of Acta Endocrinologica from 1948 to 1954 and chairman of the Norwegian Society of Endocrinology from 1948 to 1954. He was president of the Second Acta Endocrinologica Congress in Oslo 1956.

From 1966 he has been the Editor for Norway in Acta Obstetricia et Gynecologica Scandinavica. He has also been chairman of the Oslo Gynecological Society and of the Norwegian Gynecological Society.

His main field of interest was gynecological endocrinology and in particular the morphology and functions of the placenta. He won his doctor's degree in 1945 on the thesis "The Placenta in experimental Interruption and Prolongation of Pregnancy". His "Studies on the Vascularization of the human Placenta" from 1953 was a major contribution to the scientific exploration of the placenta. Its illustrations have been reproduced in standard textbooks and survey articles. Continued research resulted in a series of articles in this journal in 1967 and 1968, a fine termination of an outstanding scientific career.

Finn Bøe loved music and was an exellent piano player. His searching mind was also revealed within this field in a remarkable book on Edvard Grieg, the genius of his native city.

During the second world war Finn Bøe actively assisted the Norwegian Underground Movement at great personal danger, an unselfish service which was typical of him.

There can be no doubt that Dr med Finn Bøe through his life and work greatly influenced and inspired Norwegian medicine. Internationally his basic scientific work has provided him a lasting remembrance. With the untimely death of Finn Bøe we, his collegues and friends, have suffered a great personal loss.

Oddmund Koller

LETTER TO THE EDITOR

Pulkkinen & Kivikoski *Changes in uterine volume following the intraamniotic injection of hyper tonic saline* (Acta Obstet Gynec Scand *50* 149 1971)

In their article Pulkkinen & Kivikoski, using amniograms and planimetry observed a 30% increase in uterine volume during the initial 3 hours after intraamniotic injection of hypertonic saline. They mention that their results differ from ours *(Obstet Gynec 31 240 1968)* using a similar technique. As we pointed out in our paper because the uterus changes its shape or contour once contractions begin, one cannot calculate uterine volume with a single AP view While it may be true that we were in error in concluding that uterine volume does not significantly change (which I still believe) the authors certainly had no right to conclude anything from repeated single view X rays but that the uterus changes its shape after hypertonic saline

In my opinion, they have added further confusion to the subject by suggesting that uterine *volume increases have something to do with the* initiation of labor in saline abortions. Several centers have tested the effects of instilling variable amounts of hypertonic saline in relationship to the amounts of amniotic fluid and to the best of my knowledge have shown, that within wide limits there is no apparent effect of overdistension of the uterus on initiation of labor

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IN VIVO DETERMINATION AND CYCLIC VARIABILITY OF INTRAUTERINE VOLUME

Ingemar Joelsson

From the Department of Obstetrics and Gynecology (Head, Professor Axel Ingelman-Sundberg) of Karolinska Institutet at Sabbatsberg Hospital, Stockholm, S eden

Abstract A description is given of hysterometry method for in vivo determinations of the pressure response to linear increments in volume of the non-pregnant human uterus. The analyses of pressure–volume diagrams obtained during consecutive phases of the menstrual cycle reveal that the volume of the uterine cavity during the secretory phase larger than during the proliferative phase by factor of 2 or more. If ovulation does not occur, however the relation between pressure and volume remains unchanged throughout the interval between two menstrual flow. I is suggested that hysterometry might prove of value for assessing the effect of hormones and pharmacologically active agents upon the myometrium.

The physiological function of hollow muscular organs has been studied mostly by determining the pressure in the cavity under more or less well defined conditions. I the case of the human uterus in vivo, acceptable recording conditions cannot really be achieved until the last part of pregnancy. The hormonal influence is then uniform, irritation from the pressure sensor is at a minimum, and its volume is of no significance. The muscular contractions occur under almost isometric conditions. Absolute values of the intra-amniotic pressure become comparable entities with respect to muscular tone and contraction intensity even when comparing different individuals.

The spontaneous motility in the nonpregnant uterus in vivo is disturbed by the pressure recording instrument, which may stimulate contractions and sensitize the tissues. Undefined intermediate stages between isotonic and isometric recordings are inevitably accepted as a basis for otherwise skillfully planned test series. The information obtained in this manner is generally not standardized before the absolute values are evaluated, sometimes resulting in contradictory conclusions.

Pressure–volume diagrams have been used extensively to study the reaction of the urinary bladder as regards the tonus of the musculature, the tendency to contract and the maximum filling volume. The method has provided valuable information about the function of the bladder muscle and its innervation and has helped in the interpretation of bladder disturbances. It therefore seemed reasonable to apply a method analogous to cystometry to the study of cyclic changes in the size of the uterine cavity.

MATERIAL AND METHODS

Ten healthy women were followed during one or more of their menstrual cycles with repeated simultaneous recordings of the intrauterine pressure and volume. The women were examined on alternate days with reference to the diameter of the external os and the occurrence and physical behaviour of the mucus in the cervical canal. Concentrations in the urine of oestrogens and of pregnanediol ere determined at intervals. Diagrams of the intrauterine pressure–volume relationship were scheduled during the proliferative phase (around day 6), during the day of ovulation and during the secretory phase (around day 23).

A thin-walled latex balloon, admitting 7 ml of fluid without pressure being generated, and manufactured to fit two channel brass sound, served as the intrauterine receptor. The pressure measurements were made using an inductance type pressure transducer together with carrier wave generator and an amplifier. The volume displacement of the pressure chamber amounted to 1 10^{-4} ml/10 mmHg pressure change. The volume of the receptor balloon could be changed linearly up or down by moving the piston of syringe, arranged in modified Harvard apparatus, and connected to the second channel in the brass sound. The piston's travel could be varied in fixed steps by means of gear box, attached to synchronous

Announcement

The IX Acta Endocrinologica Congress will be held in Oslo June 17th – 21st 1973

President: Dr Jørgen H. Vogt, Medical Department B Aker Hospital, Oslo 5

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Enquiries should be addressed to the Secretary General.

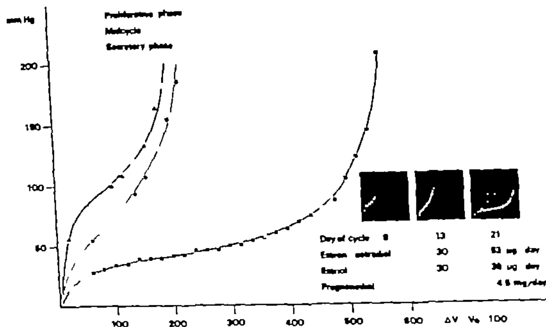


Fig. 2. Result of hysterometry on days 8, 13 and 21 in an ovulatory cycle in a nulliparous woman aged 24. At 200 mmHg intracavitary pressure the intracavitary volume is much the same in the early proliferative phase as at ovulation. The volume of the cavity during the secretory phase is 2.7 times larger than during the proliferative phase.

in pressure during muscular distension (inflation) and the descending limb the decrease in pressure during muscular relaxation (deflation). The width of the area between the limbs represents the effect of combined hysteresis of the musculature and the pparatus. It will vary with the amplitude the rate of change and the history of distension. The analysis in this study is confined to the ascending limbs of stable curves, recorded after 3 or more complete inflation–deflation cycles.

The volume of the part of the empty receptor that was actually introduced into the individual uterine cavity was calculated, using data from the anatomical measurements. The start of each hysterometry tracing was zeroed to this basic receptor olume, V. In the figures, the X axis is graduated in relative volume increments, $(\Delta V/V_0)$ 100. Pressure is indicated along the Y-axis in mmHg.

RESULTS

Examples of series of hysterometric recordings obtained in nulliparous women during ovulatory cycles are given in Figs. 1 and 2. Recordings in parous women during ovulatory cycles are reproduced in Figs. 3 and 4. A series of recordings in a nulliparous woman during an anovulatory cycle is depicted in Fig. 5. Facsimiles of the original recordings are included in all the illustrations.

The chief characteristic of the ascending limb of the hysterometric curve is a steep initial rise in pressure for small increments in volume followed by a flattening out to a more horizontal course. In the latter part of the curve there is again a rapid increase in pressure in response to small increments in volume. The three portions of the curve are more distinct in the parous than in the nulliparous women. Comparing the volumes of the uterine cavities at equivalent final pressures in the separate phases of the menstrual cycle, it will be noted that in most cases, the volume in the secretory phase exceeds the volume in the proliferative phase by a factor of 2 to 3. The limbs of the tracings recorded during ovulation (the day of ovulation plus or minus 1–2 days)

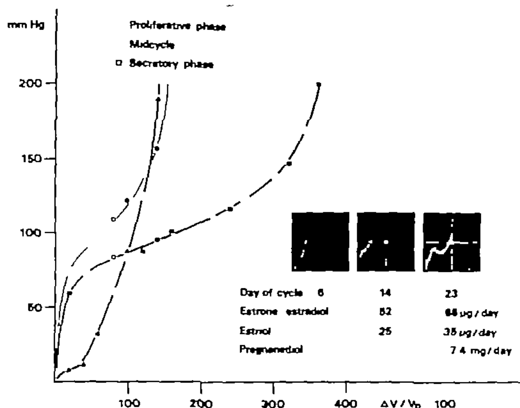


Fig 1 Ascending limbs of pressure–volume curves on days 6, 14 and 23 of the ovulatory menstrual cycle of a multiparous woman, aged 37. The volume at an intrauterine pressure of 200 mmHg is 2.5 times larger during the secretory phase than during the proliferative phase. The volume-pressure relation during ovulation is similar to that on day 6.

motor. The speed selected for filling and for emptying the receptor balloon was 25 $\mu l/sec$ to avoid the influence of spontaneous activity as well as a marked hysteresis. The pressure transducer was always connected via the channel to the proximal opening in the balloon and the unit for volume change via the channel to the distal opening. This minimized the risk of the pressure line being occluded by the rubber wall while fluid was being withdrawn. Polyethylene tubing of low compliance was chosen for the connections. The smallest inner diameter of the pressure and volume lines was 1.4 mm.

Changes in pressure and volume were documented by means of a dual beam oscilloscope and a Polaroid-Land camera. The signal from the pressure transducer was fed to the input of the oscilloscope's vertical amplifier; the impulse of the volume transducer (a multi-winding precision potentiometer operated by 6 volt D.C.), which translated the movements of the piston in the syringe, was fed to the horizontal amplifier.

Calibration of the pressure line was performed for hydrostatic pressures between 0–300 mmHg; the response was observed to be linear. Calibration of the volume line was performed for consecutive ml of infused fluid, maximum 6 ml.

The syringe was calibrated by serial weighing at standard temperature and pressure, after drawing up incremental volumes of distilled water.

Before making the hysterometric measurements, data were obtained on the geometry of the individual uterus. The distance between the external and internal os was measured by means of a number 5 Hegar dilator graduated in 0.5 cm. The distance between the level of the vaginal portio and the myometrium at the uterine fundus was established by means of a uterine sound. The depth figures were borne in mind when it came to evaluating the hysterometry curves. The receptor unit, with the balloon empty, was introduced to its proper place in the uterus without dilatation of the cervix and without anaesthesia. Following this, the patient was arranged in a relaxed and comfortable supine position. A 20-minute accommodation period was allowed before starting any recording.

After only a small number of hysterometry recordings it was clear that the subjects almost invariably experienced pain when distension of the myometrium induced a pressure of more than 200 mmHg. As it was felt that pain or discomfort would invalidate the results, the pressure-volume ratio could not be followed beyond a maximum of 200 mmHg, irrespective of the absolute value of the filling volume.

EVALUATION OF THE CURVES OBTAINED BY HYSTEROMETRY

The complete hysterometric curve comprises two limbs. The ascending limb represents the change

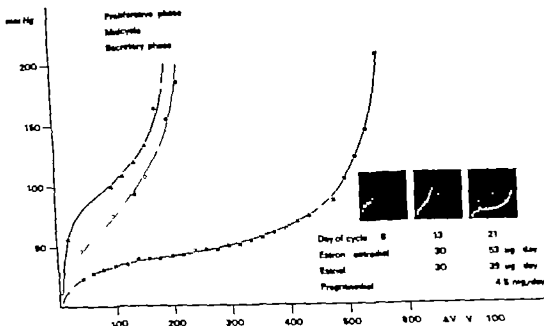


Fig. 2. Result of hysterometry on days 8, 13 and 21 in an ovulatory cycle in a nulliparous woman aged 24. At 200 mmHg intracavitary pressure the intracavitary volume is much the same in the early proliferative phase as at ovulation. The volume of the cavity during the secretory phase is 2.7 times larger than during the proliferative phase.

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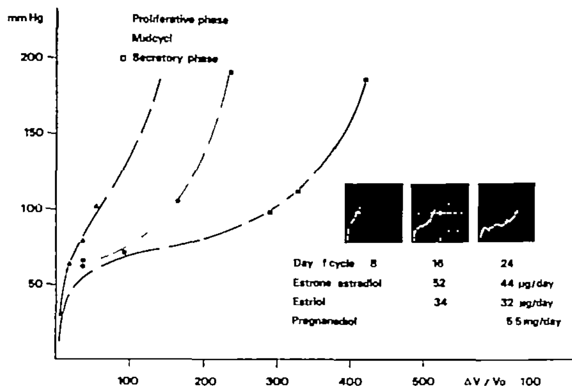


Fig 3 Pressure–volume diagrams during consecutive phases of an ovulatory cycle in a primiparous woman, aged 27 The uterine volume on day 4 is 1 7 times larger than on the day of ovulation and 2.9 times larger than on day 8.

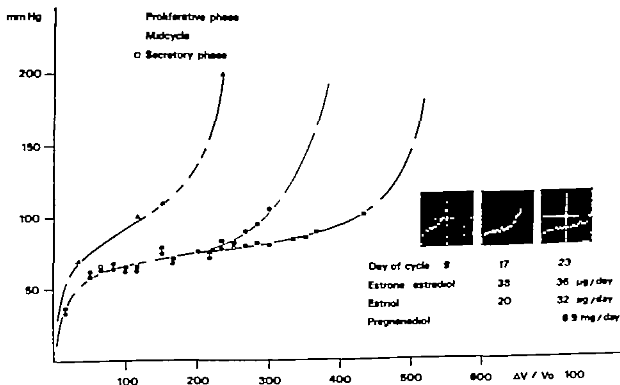


Fig 4 Hysterometric recordings during an ovulatory cycle in a secundoparous woman, 39 years old. At similar pressures the uterine volume during the secretory phase is 1.4 times larger than during ovulation and 2.3 times larger than during the proliferative phase.

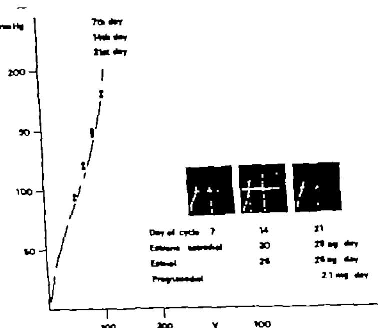


Fig 5 Intrauterine pressure-volume diagram on three different occasions during an anovulatory cycle in multiparous woman, aged 37

closely resemble those from the proliferative phase in the multiparous women but lie between the limbs from the proliferative and the secretory phases in the case of the parous women.

In one of the women, ovulation did not occur. Here it will be observed that the ascending limbs of the curves at day 7, day 14, and day 21 are exactly alike.

For technical reasons related to the construction of the apparatus it was not always possible to achieve the same final pressures in the recordings during the secretory phases as in those during the earlier phases of the menstrual cycles.

DISCUSSION

The mathematical equation relating the internal pressure to the radius of sphere, shows that the numerical value assigned to a single constant characteristically determines the shape of the corresponding curve (2). A family of curves, constructed for arbitrary values of this constant, will display an impressive similarity to, for instance, cystometry curves published by Langworthy, Reeves & Tauber (3) following recordings in the cat in various phases of surgical procedures and their sequelae. The same situation is mirrored by the cystometry curves published by Simeone & Lampson (4), representing the pressure-volume relationship of the urinary bladder under normal conditions, in connection with obstruction of the urethra and influenced by disturbed innervation.

In this study it was observed that the hysterometric curves, relating pressure to volume of the irregular cavity of the thickwalled uterus during the course of the menstrual cycle, resemble a set of curves for pressure–volume relationships in general. The parameter which determines the shape of these curves may, however, reflect the combined effect of several phenomena. Most probably one of these phenomena represents anatomical changes in the muscle cells and the muscle fibrils. Longitudinal growth of the cells of the uterine myometrium is known from animal experiments to be associated with oestrogenic stimulation. There is also reason to believe that links between muscular fibrils do occur and do vary in strength with hormonal influences. Strong links of this type might stabilize the myometrium, producing a shift of the pressure–volume curve to the left. Another phenomenon might mirror the elastic condition of the connective tissue, which is integrated with the musculature. The elasticity of the ground substance changes as the individual

grows older (1) and it might therefore be of interest to record the uterine pressure–volume relation during a menstrual cycle repeatedly in the same woman at yearly intervals.

Hysterometric curves of the type discussed here should be recorded with due consideration to the speed of a filling and emptying cycle. If the volume is changed only slowly the hysteresis will be insignificant. The pressure–volume curve will reflect the muscular activity which represents the tone amplitude and frequency of contraction that the organ adopts at a certain level of distension The shape of curves obtained with a slow rate of enforced distension becomes constant after only 2 or 3 cycles. If the volume is changed rapidly however the hysteresis phenomenon will be marked and the shape of the ascending limb will vary for a number of consecutive loops until stable conditions prevail.

In this study of the relation between pressure and volume of the uterine myometrium it has been observed that individual characteristics are detectable in nulliparous and parous women. The influences upon the myometrium effected by the function of the normal ovary seemed to be marked. During ovulatory cycles the volume of the cavity in the secretory phase at equivalent pressures, was at least twice the volume during the proliferative phase. In parous women the pressure–volume relation during ovulation was markedly shifted to the right, while in nulliparous women it was similar to that during the proliferative phase.

It would be of interest to analyse curves obtained under experimental conditions with artificial control of hormonal influences. In addition, recordings in conjunction with the administration of pharmacologically active contractile or relaxant agents might provide information on the effect of drugs information that might prove even more useful than the conventional parameters tone, amplitude and frequency of contractions.

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ULTRASONIC LOCALIZATION OF INTRAUTERINE CONTRACEPTIVE DEVICES

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Abstract. Thirty women were examined by ultrasonic scanning before and immediately after the insertion of an intrauterine contraceptive device (IUCD). Fifteen of them had the Lippes loop inserted, ten the coil (Gynecoil®), and five the steel band (Inhiband®). Both the polyethylene and metallic contraceptive devices were well visualized by ultrasound, and each gave an echo image corresponding to its shape. In addition to this, a woman who had, in spite of the Lippes loop, become pregnant and whose pregnancy was in the eighth week, counted from the beginning of her last menstruation, was examined. The ring-like gestational sac could be seen in the inside of the normal uterus. Further, the echo image of the Lippes loop was obtained from the inside of the uterus. The uterine size and the size of the gestational sac corresponded to the stage of the pregnancy as indicated by the patient.

The use of IUCDs as a method of preventing pregnancy is becoming more and more popular. Many women who earlier resorted to the Pill have asked their physician about this method. A great number of contraceptive devices are available. Ishihama (2) and Oppenheimer's (4) favourable observations in 1959 have inspired experiments with new materials and types. Most of the IUCDs employed have been made out of barium-impregnated polyethylene. In addition to these, metallic devices made out of rustproof steel or copper are used. The most popular have been the Lippes loop, the Margulies spiral, the Birnberg bow, the Hall-Stone steel band and the double coil (Saf T-Coil).

The IUCD prevents pregnancy only when it is in place in the uterine cavity. Unfortunately with some women it does not remain inside the uterus, but is constantly expelled into the vagina. It has been established that spontaneous expulsion of the IUCD occurs in 10–20% of the women employing the device. Expulsion is most common during the first five menstrual cycles, generally during menstruation. It has been established that the coils are expelled more often than the loops (5). It is customary to advise women to check after menstruation whether the markers or the tail are recognizable, and if not, to consult the physician. If the markers cannot be found, even in a gynecological examination, it is possible that the contraceptive device has been expelled from the uterus. Its markers may for instance have become entangled in the sanitary tampon and the device may have been pulled out when the tampon has been removed. It is possible, too, that markers which have been cut too short may have withdrawn out of sight into the cervical canal or they may even have broken loose from the device. A more serious complication exists if the IUCD has perforated the uterine wall, partly or completely. It is difficult to determine the exact frequency of perforations because most cases are asymptomatic. The usual estimation has been 1 perforation per 1 000 insertions. However, Ledger & Willson (3) for example, stated in 1966 a frequency as high as 5 cases per 2 000 insertions for coils and loops. It is probable that in most cases the migration of the contraceptive device into the peritoneal cavity has been caused by uterine perforation at the time of insertion. This assumption is among other things supported by the fact that the contraceptive device is found in the peritoneal cavity more frequently in those women who had the insertion performed at puerperium. When controlling the position of the IUCD, a special problem is created by those types which from the very beginning have no markers.

When expulsion of the IUCD is suspected, a method of examination which has been used up

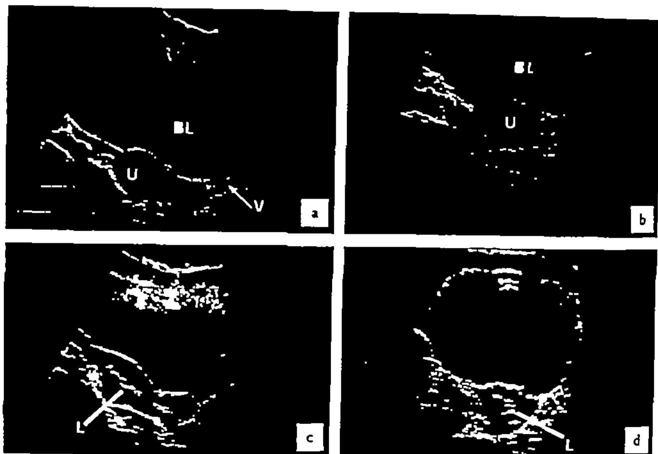


Fig 1 a, b and *d*. A longitudinal section of the uterus ( ) and transverse section (*b*) before the insertion of the contraceptive device. *Bl*, bladder *U* uterus, *V* vagina. A longitudinal section of the uterus (*c*) and a transverse section (*d*) after the insertion of the Lippes loop (*L*).

to the present time has been to examine the uterine cavity with a probe. The findings are, however unreliable and the procedure involves, even in sterile conditions, the risk of infection. For this reason X ray films have generally been used. Even the polyethylene devices are, because of their barium content, roentgen positive. It is not, however possible to establish with a plain film whether the contraceptive device visible in the pelvis is in an intrauterine or extrauterine position. Besides, ionizing radiation has to be directed towards the gonads of the woman. Exceptionally the case might involve an undetected early pregnancy where the radiodiagnosis constitutes a danger to the fetus.

Diagnostic ultrasound is a new method of locating the IUCD Because of the different nature of its energy it does not like radiography endanger the patient or her eventual gravidity The purpose of this publication is to draw attention to a new method of locating the IUCD which has not been presented earlier with ultrasonic pictures.[1]

MATERIAL AND METHODS

Thirty women who wanted an IUCD were examined at the Womens Clinic of the Central Hospital of Turku University by ultrasonic B-scanning before and immediately after the insertion of the contracepti e device at the clinic. All women had had one or more deliveries. The patients had been chosen so that in their previous menstrual history or gynecological status there was nothing contra-indicating the insertion of the IUCD Further, the B-scanning f the uterus was performed on 27-year-old woman, G_2P who had come to the hospital for legal abortion and who 8 months before had had the Lippes loop inserted, in spite of which she had become pregnant. At the time f the examination 8 weeks had passed from the beginning of her last menstrual period.

In order to enable the uterus and the organs in its vicinity to be visualized in the ultrasonic examination, the "full bladder technique was used. Before the insertion the uterine position and size were established by B-scanning, and attention was paid to uterine anomalies and uterine or adnexal tumours (Fig. 1 *a* and *b*). The uterine

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During the printing of this publication the author has been informed about the letter of Nemes and Kerenzi to the edito concerning the ultrasonic localization of IUCDs. Amer J Obstet Gynec *109* 1219 1971



Fig. 2. The intrauterine contraceptive devices used in the study: the Lippes loop, the coil (Gynekoil®), and the steel band (Inhiband®) from the left to the right.

size as established from the longitudinal section of the uterus by taking the longitudinal measurement and the greatest AP-diameter (Fig. 1 *a*). The examination was carried out with ultrasonic equipment constructed by the Kretztechnik-factory (Serie 4100 MG-8, Kretztechnik, Zipf, Austria), using in most cases 2 MHz crystal and olive oil as the contact medium between the skin and the probe. The contraceptive devices employed in the examination were the Lippes loop and the intrauterine coil (Gynekoil®), both of which are synthetic and the rust-proof steel band (Inhiband®) (Fig. 2). The last mentioned consists of 3 laminated 0.013 mm thick flexible rings, which are made to a spring coil. Fifteen of the women examined had the Lippes loop inserted, ten the coil and five the steel band.

RESULTS

By the B-scanning before the insertion of the contraceptive device the uteri of all 30 women were established to be normal. The uterine size varied individually and depending on parity.

With two exceptions the contraceptive device could be well seen in the uterine cavity after the insertion. In two cases (1 loop and 1 coil) the patient complained of lower abdominal pains and no immediate B-scans could be obtained. An examination a day later proved that the contraceptive device was in these cases, also in its proper place in the uterus. The Lippes loop could be seen in the longitudinal section as a broken longitudinal line. Sometimes the echoes from the loop formed a nearly continuous line. In the transverse section of the uterus the loop gave a ladder-like echo image where transverse echo lines of the uterine cavity could be seen, one below the other (Fig. 1 *c* and *d*). The intrauterine coil (Gynekoil®) could be seen in both the longitudinal and transverse section as an echo image, resembling the Lippes loop (Fig. 3). The steel band (Inhiband®) was visualized in the longitudinal B-scan of the uterus either as an oval ring or as a longitudinal line corresponding to its size, depending on the directional angle of the ultrasonic beam. In the transverse section of the uterus the steel band was seen, either as a ring-like echo image or as a transverse line in the uterine cavity (Fig. 4).

In the B-scanning of the patient who had become pregnant in spite of the Lippes loop, the ring-like gestational sac was confirmed in the fundus of the uterine cavity. In addition to this, both the longitudinal and the transverse scanning gave, from inside the uterus, the typical echo





Fig. 3 a and *b*. A longitudinal section of the uterus (*a*) and a transverse section (*b*) after the insertion of the coil (C).









Fig 4 a, b c and d The sonogram of the steel band (*B*) in a longitudinal section of the uterus (and), and in transverse section (*b* and *d*).

image of the Lippes loop (Fig 5) The average diameter of the gestational sac was 3 cm. The longitudinal measurement of the uterus was 12 cm and the greatest AP-diameter 6 cm. One day later evacuation of the uterine cavity was per formed as a legal abortion and simultaneously the Lippes loop was removed

DISCUSSION

In cases where there has been uncertainty as to the location of the contraceptive device, examination of the uterine cavity with a probe or radiography have been methods employed previously The disadvantage of the probe method lies in the unreliability of the findings and the risk of infec





Fig 5 and *b* A pregnancy at 8 weeks, occurred in spite f the Lippes loop. In a longitudinal section of the uterus and in *b* transverse section of the uterus. *GS* gestational sac

tion. On the other hand, radiography involves the use of ionizing energy. A new method of locating the IUCD is the ultrasonic examination by B-scanning. Both the polyethylene and metallic contraceptive devices show up well in the uterine cavity and give an echo image corresponding to their shape. If the IUCD is completely perforated into the abdominal cavity its ultrasonic localization between the loops of the intestines may be difficult or impossible. In this case the radiography should still be used. Ultrasonic examination would be particularly useful before the insertion of the IUCD and immediately afterwards. The position of the uterus can be ascertained, its size defined, uterine anomalies and tumours diagnosed, the location of the contraceptive device after the insertion established, and a perforation revealed in this way. In exceptional cases B-scanning is not successful immediately after insertion. The procedure may have caused cramp-like contractions which disturb the ultrasonic examination. Even in these cases the examination may be successfully completed, generally one day later.

If pregnancy has occurred in spite of the IUCD the location of the contraceptive device may be established by B-scanning and the exact stage of pregnancy defined (1) without endangering the fetus.

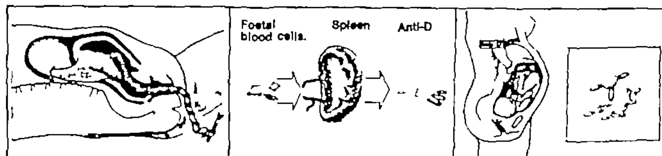
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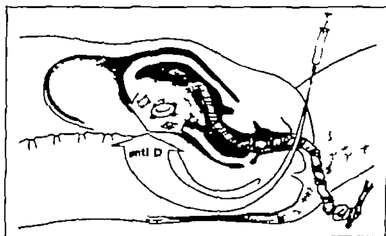
Prevention of Rh-immunization



Schematic illustration of how a small amount of blood crosses over from the foetus to the mother during parturition.

Foetal Rh-positive blood cells elicit the formation of antibodies in the Rh-negative mother

Antibodies pass over from the Rh-immunized mother to the foetus



Prophylactic administration of Gammaglobulin anti-D to the mother within 72 hrs. after delivery in order to prevent Rh-immunization

Gammaglobulin anti-D Kabi

without mercury Storage refrigerator Shelf life 3 years.
Pack 250µg ampoules, comb package



Acta Obstet Gynec Scand 51 209–215 1972

A FLOWMETER FOR THE DETERMINATION OF SMALL FLOW RATES

Ingemar Joelsson and Erik Odeblad

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Abstract. A method, capable of determining very small flow rates, 0.01–0.10 µl sec^{-1} is described. The technique, which is devised with the aim of measuring the rate and fluctuation in the flow of uterine secretions, is based upon the detection of the asymmetry in the dispersion of heat, added as a label. The accuracy in measurement of basal secretion rates over long periods of time is ±15%, the accuracy in the detection of flow fluctuations is ±5%.

Preliminary data on the flow of secretions through the cervical canal has been presented in a previous report by Enhörning, Ingelman-Sundberg & Joelsson (4). A glass capillary tube was used for this purpose, the secretions being collected at the external os of the uterus in a polymethyl-methacrylate chamber which adhered to the vaginal cervix by suction, and a transmission system linked the receptor to the recording device (Fig. 1). This method was suitable for the measurement of basal flow rate. The fluctuations of flow that were observed were shown to be due to uterine motility as well as to changes in intra-abdominal pressure. It was thought that with a modification of the method, the flow of secretions through the cervix could be used to detect only variations in the volume of the uterus.

The new method for flow measurement was developed in the light of experience gained with the capillary tube system. It was concluded that the receptor, preferably a cup for collecting secretions combined with a transducer, should be sufficiently small to fit in its entirety into the upper part of the vagina without causing discomfort. It was assumed that if the detecting device had an open end in the posterior vaginal fornix, this would partly compensate for variations due to changes in abdominal pressure. Furthermore, a short receptor canal and no transmitting system would minimize the interference with flow recording, attributable to the anomalous behaviour of the secreted mucus (1, 2, 3). The method should permit estimation of flow rates even if a label is added to the liquid column in only minimal amounts in order to prevent the chemical (7, 9, 10) and physical (5 with references, 6) properties of the substance from being altered to a significant degree.

Detection of Asymmetrical Dispersion of Heat

The heat from a filament fitted in a horizontal column of a liquid is chiefly dispersed by diffusion, which is equal on both sides of the heater. The temperature gradient in the lateral directions is mainly a function of the diffusion constant.

Provided the liquid column does not move and there are no convection currents in the liquid, two thermometers placed symmetrically around the source of heat input will be exposed to identical amounts of heat. If the liquid is in motion, however, the thermometers will be in zones of different temperature. At low flow rates, the added heat will not be diluted enough to outweigh the preferential dispersion of heat in the direction of flow.

Thermistors are semiconductors, characterized by temperature-dependent resistance, and they are a useful tool for the detection of small temperature differences if connected in a bridge fed an adequate electrical impulse. The Wheatstone bridge could be used for this purpose, but the symmetrical T-network described by Tuttle (8) has certain advantages in that its earth connection is common to the input and output sides of

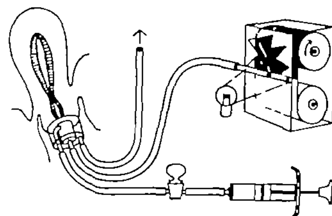


Fig 1 Receptor of the capillary tube method for measurement of secretory flow through the cervical canal. The inner cylinder is used for collecting the secretions, it can be filled through the side arm to the left. The side arm to the right is used for connecting the space between the cylinders to a low pressure source. In the transmitting system a drop of mercury is placed in contact with the liquid column and over the film track.

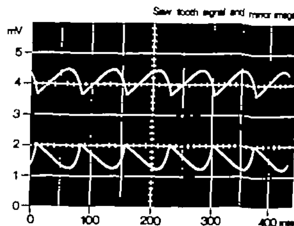


Fig 3 A saw-tooth type signal and its mirror image produced by a phase splitter and displayed on the oscilloscope.

the bridge. Provided the absolute resistance at a given temperature and the change in resistance per degree centrigrade are both identical for two thermistors within the temperature range employed, these can be connected in the twin T bridge circuit as indicated in Fig. 2. Most often the temperature of the thermistor is determined by the thermal state of the environment since the heat output from the thermistor itself is restricted

Frequently it is important to determine not only the rate of flow and its variations but also the direction of movement of the liquid To achieve this, the two input branches of the bridge must be activated in a specific way This can be done using synchronously a saw-tooth type signal

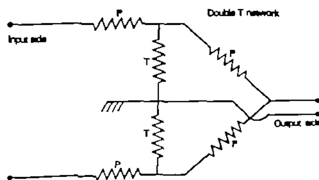


Fig 2 A double T-network. The resistances *P* in the input and output arms represent matched precision resistors, the resistances *T* represent the temperature-dependent resistors (thermistors).

and its mirror image (Fig. 3) if there is no flow of liquid, the two input signals will counter balance when flow occurs there will be an output signal, the amplitude of which will be a function of the flow rate reversed direction of flow will cause the mirror image to gain predominance.

In order to record the output signal on the bridge, the impulse has to be amplified. A conventional recorder may then be attached through a cathode follower coupling.

THE APPARATUS

The receptor unit

Collection of uterine secretions was achieved by applying a polymethyl methacrylate chamber to the vaginal cervix. The external cylinder of the receptor was fitted with a terminal base plate. A tube, of similar plastic, with 3 mm inner diameter and 12 mm long acted as a central drainage channel for the chamber collecting the secretions A heat source and a pair of thermistors were mounted in this tube. Polymethyl-methacrylate tubing was also used for a side channel for filling the collecting chamber with liquid and for the channel for connecting the vacuum pump to the receptor (Fig. 4)

Induction of a change of heat content in the secretions is achieved by passing an electric current through a high resistance nichrome wire which forms the heat source The wire used in this case had a diameter of 0.013 mm giving a resistance of 1 1 ohms/cm at a temperature of

37 C. The nichrome wire was coated with tin where it pierced the drainage tube in order to minimize heating of the plastic tubing surrounding the liquid. The current needed was obtained from a power supply which was designed to meet the specific requirements.

Thermistors alter their electrical resistance upon changes in temperature, $R = f(T)$. The resistance change can be either positive or negative to the change in temperature. Negative temperature coefficient (NTC) thermistors are those generally employed. The change in resistance in relation to change in temperature for a thermistor can be expressed in a variety of ways. Most often it is given as the percentage change in resistance per degree centigrade. This value is known as the temperature coefficient of the thermistor. For those employed in this study the temperature coefficient is in the range of -3% to -4% per degree centigrade at 20°C.

An approximation of the relationship between resistance and temperature for thermistors is given by the following equation: $R = R_0\, e^{B/T}$ where R is the resistance of the thermistor, e is the base of the natural logarithm, R_0 and B are constants, and T the temperature in degrees centigrade. The constant B varies from thermistor to thermistor even if they belong to the same production series. This is attributed to minor differences in the chemical constitution of the semiconductor material and to differences in distance between the wire leads. Thermistors to be employed in pairs in a bridge circuit, therefore, have to be selected among those having similar characteristics. This requirement made it necessary to carry out a special study.

The true resistance values at different temperatures between 35 and 45°C were determined for a number of thermistors. The thermistors were connected to a switchboard on the grid input side of a double triode in a cathode follower coupling (Fig. 5). The thermistor beads were immersed in a thermostatically controlled oil bath maintained within ± 0.02 C of a predetermined temperature. Outgoing impulses from the tube were displayed on an oscilloscope. Pairs of thermistors showing identical temperature coefficients were selected for use in the flowmeter.

The properties of a thermistor sometimes have to be modified. This is true particularly when working with pairs of thermistors, even if they have been matched. The modification cannot be done by changing the chemical or physical properties of the semiconductor. It can instead be effected by combining the thermistor with a resistor or linear potentiometer in a shunt circuit. Thereby, however, the temperature coefficient of the combined system becomes reduced.

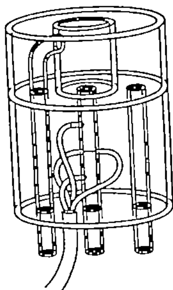


Fig. 4. The receptor unit of the flowmeter based on detection of differential dispersion of thermal energy. The thermistors are placed symmetrically around the heater in the central channel, which constitutes the outlet for the secretions.

The bridge circuit

The wiring for the twin-T bridge was carried out as shown in Fig. 2. Testing the bridge showed that with this arrangement heating of a stationary column of liquid in a horizontal channel created an imbalance on the output side of the bridge circuit. This may be due to differences in the thickness of the glass covering the thermistor beads and to minute differences in the distance of the thermistors from the heat source. When the longitudinal axis of the transducer channel deviated from the horizontal plane in either direction (Fig. 6) the imbalance was accentuated because of convection in the liquid column. In order to minimize the influence of these variables upon the measurement of flow a linear variable resistor of the carbon potentiometer type was used as a shunt with each thermistor in accordance

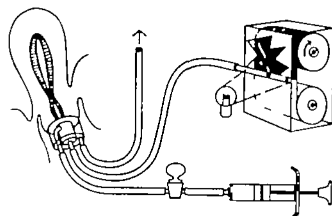


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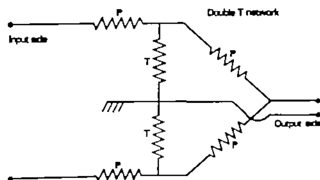


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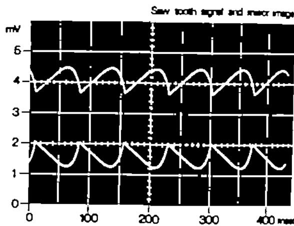


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thermistors is adjusted to be the same, there would be a balanced output signal from the phase-splitter. However non-linear functioning of the components of the phase-splitter produces curves with upward and downward convexities between the impulses. This non-linear function can be compensated with components of a defined non-linearity. Varistors are a subgroup of such resistors of semi-conductor material, the resistance of which decreases as the applied voltage increases. After the introduction of varistors in the anode and the cathode circuits of the phase inverter tube, it was found that compensation of non-linearity under service conditions required a variable varistor effect. This was obtained by connecting the varistor in the circuit in parallel with a linear potentiometer. In the phase-splitter however it is necessary to maintain the overall resistance of the anode and cathode circuits unchanged, irrespective of the variations in the potentiometer setting, in order not to disturb the symmetry of the outgoing signal. To attain this, an additional linear potentiometer the resistance of which varied inversely with that of the shunting potentiometer was placed in series in the principal circuit. A detailed wiring diagram for the anode and cathode circuits of the phase inverter tube in accordance with these modifications is given in Fig. 10.

The amplifier

The variations in potential of the output of the bridge circuit require considerable amplification for recording purposes. Realizing that the untoward effect of the capacitance of condensers should be avoided a two-stage D.C. amplifier with

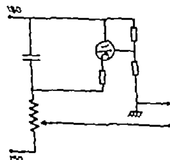


Fig. 8. Impulse generator using thyratron. The frequency of impulses is controlled by capacitor, connected in parallel with the tube.

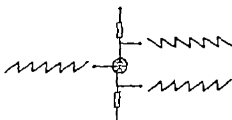


Fig. 9. The synchronous splitting of the generated impulse into its mirror-inverted components is accomplished by using triode connected as a cathode follower.

direct connection was constructed. After gradual adjustment of the incorporated components a maximum power amplification of 1 000 times was obtained. The amplifier was connected to a cathode follower in accordance with the wiring in Fig. 11.

The recorder

A recorder with an electromagnetically operated pen (Brush) was selected. This gave a linear response over a deflection range of 12 mm.

Performance Characteristics of the Apparatus

After tests with varying amounts of heat added to the cervical secretions it was found that a heat output of 4 10⁻ cal sec⁻¹ provided the most

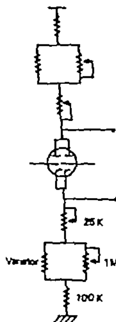


Fig. 10. Wiring diagram for the anode and cathode circuits of the phase inverter after introduction of varistor and two linear potentiometers, the resistance of which can be varied inversely in order to maintain the overall resistance of the circuits.

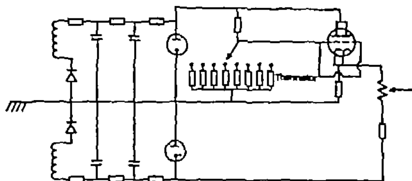


Fig 5 The circuit allows thermistors to be individually connected between earth and the grid input side of a double triode. The outgoing impulse can be calibrated to indicate the resistance of the thermistor in relation to actual temperature.

with the wiring diagram in Fig. 7 This arrangement made it possible to balance out any signal from the bridge after the transducer had been fastened to the cervix, flow restrained and heat added to the liquid.

The impulse generator and phase splitter

The impulses feeding the twin-T bridge should be of a saw-tooth type. Such impulses can be generated using special tubes known as thyratrons. The frequency of impulses is controlled by a capacitor connected in parallel with the anode and cathode of the tube. The driving voltage to the tube is supplied from a mains transformer rectified, smoothed and stabilized. The diagram of this circuit is given in Fig. 8.

The synchronous splitting of the generated impulse into two mirror-inverted components which can feed the two arms of the input side of the twin-T bridge is accomplished by using a triode connected as a cathode follower The principle underlying phase reversal of this kind is illustrated in Fig. 9 The anode and cathode of the triode are connected between a stabilized potential and earth over identical resistances. The conductance of the triode is controlled by the voltage applied to the grid. The signal thus applied to the grid appears in the same phase at the cathode resistor and in the inverted phase at the anode resistor

The signals transmitted by the phase inverter were studied in detail using a dual beam oscilloscope. The impulse from the generator was contrasted with the anode and cathode signals of the phase-splitter which in turn were studied simultaneously with a balanced output signal from the bridge. It was found that the anode and cathode signals differed to some extent, causing distortion of the signal from the output side of the bridge circuit. This may be attributed to a number of factors, such as the presence of grid current, a lack of constancy in the anode voltage and variations in the resistance values in the high stability resistors.

Assuming that the thermal dissipation is symmetrical and not influenced by dilution or preferential gains (the flow of secretions through the receptor is 0) and that the sensitivity of the 2

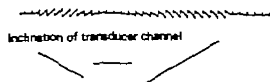


Fig 6. Deviation of the longitudinal axis of the flow rate transducer from the horizontal plane in either direction causes an imbalance of the output signal from the bridge. This is ascribed to convection flow in the liquid column.

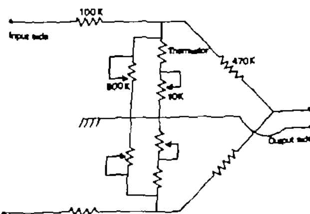


Fig 7 The final wiring of the bridge circuit. Linear variable resistors of the carbon potentiometer type are used to shunt the thermistors. This arrangement makes it possible to balance out any signal from the bridge circuit after flow has been restrained.

that maximum changes of flow rate were recorded within 4 seconds, giving a slope value of 0.014 μl sec⁻¹. The performance of the flow meter was not affected by reversal in the direction of flow. The implication of these observations is that the flowmeter is suitable for detecting rapidly occurring changes in the flow of uterine secretions such as due to motility but that it is less satisfactory for the evaluation of secretory rates over prolonged periods.

DISCUSSION

This technique for the measurement of flow rates of 0.01–0.10 μl sec⁻¹ has similarities with the thermostrom-Uhr of Rein. When the principle is applied to the very slow flow of uterine secretions, however, it is the yield in heat due to preferential thermal dispersion in the direction of flow that is detected, and not the dilution of the added heat. It has been shown that when the heat input amounts to $4 \cdot 10^{-4}$ cal sec⁻¹, the difference in temperature between the warm and the cold thermistor in the receptor unit is linearly proportional to the rate of flow.

The method can be said to have an accuracy of about ±15% from the calibration curve and the 95% confidence limits computed from long-term measurements of the given flow rates. During short periods of recording (up to 10 min), however, the maximum difference in amplitude for a given rate of flow did not exceed 5%. The electrical instability in long-term recordings is to be expected in view of the characteristics of the electrical components. Supposing the temperature difference between the thermometers is 0.5 C and the temperature coefficient of the thermistors is −3%, the change in resistance of one thermistor due to the base line flow amounts to about 350 ohms. In comparison, the precision resistors in the signal and T-network circuits are stable only at the level of ±1%. It is appreciated, therefore, that variations of the resistance of these resistors can have a significant influence on the result.

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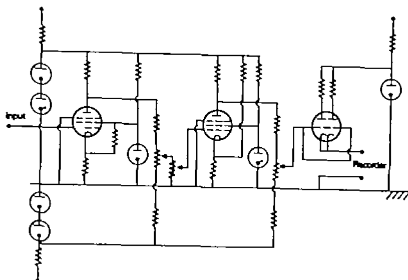


Fig 11 Two stage D C.-amplifier with direct connection. The amplifier is connected to the cathode follower and the recorder an electromagnetically operated pen.

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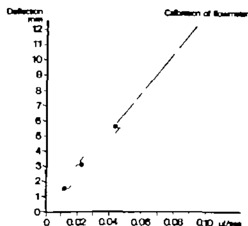


Fig 12 Relation between known flow rates and pen deflection, each plotted value (closed circle) represents mean of 100 amplitude determinations The dotted lines represent the 95% confidence limit, corresponding to 1.96 standard deviations.

obtained in the calibration studies are summarized in Fig. 12. Each plotted value represents a mean of 100 amplitude determinations. The knowledge of the standard deviation of the observed amplitude for given flow rate enables one to estimate the confidence interval for an unknown flow for a given pen deflection. Accepting a 95% confidence limit, corresponding to 1.96 standard deviations, a pen deflection of e.g. 4 mm will correspond to a flow rate of 0 026 μl sec [1] to 0 034 μl sec [1] Although these variations are considerable, it must be pointed out that in this experiment the data were collected over a long time period (up to 5 hours) and that the reproducibility for a given flow rate over a short time interval was considerably better It was observed that the maximum difference in the tracing at a constant flow rate over a 10 minute period did not exceed 5%

The estimation of the time constant was initially attempted from the records of flow rates delivered by the infusion pump operating at various velocities. In these experiments the 50% level of the ultimately observed flow rate was reached within 10 seconds, corresponding to a slope of 0.006 μl sec^{-2} This appeared to be due to the tolerances in the mechanical components of the system such as the gear trains and the syringe connections. Thus the response time was derived from data obtained in clinical application of the flowmeter In these studies it was found

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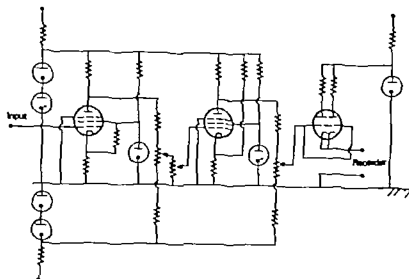


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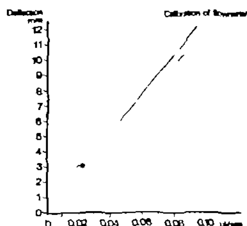


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POST TERM PREGNANCY

A Five-Year Review from Osaka National Hospital

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Abstract. The effects of post-term pregnancy on fetal and neonatal survival have been analyzed in 5 596 single pregnancies, including 275 post-term pregnancies, during the five-year period from January 1, 1962 to December 31, 1966 at the Department of Obstetrics and Gynaecology, Osaka National Hospital. The perinatal mortality rate at term was 0.7% and post-term was 2.2%. The increased perinatal mortality characteristic of post-term pregnancy could not be explained. No significant improvement in fetal outcome is achieved by induction of labour in post-term pregnancy. It would not appear to be justifiable, on the strength of the data presented here, to perform induction of labour solely because pregnancy is prolonged or in the absence of other generally accepted obstetrical indications.

Post-term pregnancy is one of the most important problems in obstetrics and has been investigated and discussed with a considerable amount of controversy especially with regard to the prognosis for both the mother and her baby.

Among unresolved problems with post-term pregnancy is an apparent increase in perinatal mortality as compared with term delivery. The fetus is exposed to a notably increased risk in prolonged pregnancy (3, 6, 10, 1.., 13, 14, 22). On the other hand, other workers have reported no differences in perinatal mortality rates between normal and prolonged pregnancies (15, 17, 18). This controversial problem is thus represented by these two directly opposing opinions.

Initially the main factors responsible for this increased fetal risk were thought to be excessive fetal size, uterine dysfunction and senility of the placenta. The significance of these factors is, however, debatable. Recent studies on birth weight in relation to the gestation period have cast doubt on the hazard of oversized babies in post-term pregnancy. The search for some indications of placental senility, as measured by increased fibrosis of the vessels, increased calcium deposits or other histological changes, has proved disappointing. The thesis that, following the predictable life span, placental function deteriorates rapidly placing the fetus increasingly in jeopardy, is attractive. In contrast, some investigators oppose this thesis.

The purpose of this study was to define the perinatal risks, if any, which are presented by the prolongation of pregnancy and to determine what factors had a significant bearing upon fetal outcome. My hope was to establish reasonable clinical guidelines for management of prolonged pregnancy.

MATERIAL AND METHODS

A total of 5 596 single pregnancies treated at the Department of Obstetrics and Gynaecology, Osaka National Hospital during the five-year period from January 1, 1962 to December 31, 1966 were examined. Twins, breech deliveries, multiple births and all deliveries before 28 weeks had previously been excluded. Also excluded from the series were those cases where the menstrual history was uncertain. From these obstetrical records such factors as duration of pregnancy, perinatal death, mean birth height, mean birth weight, mean circumference of the head, character and duration of labour and induction of labour were studied. The results obtained were analyzed statistically chiefly by the χ^2-test.

RESULTS

Duration of pregnancy

According to Naegele's rule for calculation of the expected date of confinement, the duration of

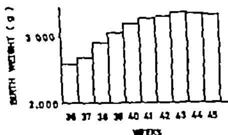


Fig. 3. The mean birth weight related to duration of pregnancy

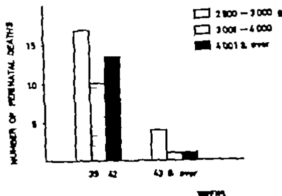


Fig. 5. Perinatal death related to birth weight and duration of pregnancy

Birth height, birth weight and circumference of the head related to duration of pregnancy

The mean birth weight, weight and circumference of the head are shown in Figs. 2, 3 and 4. These three means showed a gradual increase at each week of gestation up to the forty-second week. After the forty-third week the mean infant birth height, weight and circumference of the head were relatively stationary and showed no remarkable increase (Figs. 2, 3 and 4).

Perinatal death related to birth weight and duration of pregnancy

It has been suggested that the increased post term perinatal mortality rate may be directly related to an increased birth height, weight and head circumference of post-term infants. This suggestion, however, might be unacceptable, since in this series the mean infant birth height, weight and circumference of the head remain stationary in prolonged pregnancy. The highest perinatal mortality rate was found for infants who weighed between 2 500 and 3 000 g both in term and post term deliveries (Fig. 5). In other words, increased infant birth weight is not always responsible for increased post-term death.

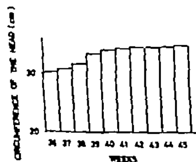


Fig. 4. The mean circumference of the head related to duration of pregnancy

Methods of delivery

The frequency of obstetrical operations such as caesarean section and forceps delivery or vacuum extraction was compared in term and post-term pregnancies. There was no difference between the two groups. The caesarean section rate was about the same: 4.1% of the term and 5.9% of the post-term. And also the rate of forceps delivery and vacuum extraction was almost the same, 2.7% of the term and 4.0% of the post-term (Tables III and IV). No statistically significant difference was found between the two groups.

Indications for caesarean section

A comparison of the more frequent indications for caesarean section at various stages of pregnancy is shown in Fig. 6. It must be noted that cephalopelvic disproportion as an indication for caesarean section does not increase in post-term pregnancy. Cervical dystocia and uterine dysfunction seem to be increasing when pregnancy is

Table III. *Caesarean section according to duration of pregnancy*

Weeks	Pregnancies	C-section	%
37–42	5 321	221	4.1
43 & over	271	16	5.9

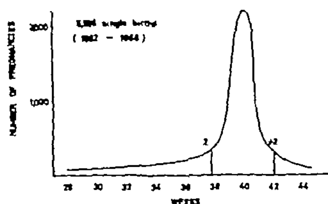


Fig 1 Duration of pregnancy

pregnancy is defined as 280 days or 40 weeks from the first day of the last menstrual period before conception. In the studies so far reported, there is some slight variation about the number of days of amenorrhoea beyond which the preg nancy should be diagnosed as "post-term" or "prolonged" Racker et al. (19), and many others have chosen those cases which gave beyond 294 days, while Eastman (7) and, a few others, only ac cepted those cases going beyond 301 days.

In the present study it was originally postulated that those patients whose date of delivery ex ceeded two standard deviations from the mean would be considered post-term" and would be studied for comparison with the cases of delivery at term. Two standard deviations above or below the mean should encompass 95% of the popula tion in a normal distribution curve. The mean duration of pregnancy in this series was 278 5 days. One standard deviation was computed to be 8.4 days, and two standard deviations 16.8 days (Fig. 1) Therefore it was concluded that pregnancy which extended beyond 295.3 days would be defined a "post-term" pregnancy in this series. This statistically based criterion is in agreement with that of many other investigators who quote

Table I *Perinatal deaths according to duration of pregnancy*

Weeks	Cases	Perinatal deaths	%
37–42	5 321	41	0.7
43 & over	275	6	2.2

The difference between these two groups is statistically significant.

Table II *Causes of perinatal deaths*

	37–42	43 & over (weeks)
Cause unknown	10	2
Anoxia caused by cord complications	6	1
Birth injury	3	0
Congenital malformations	8	2
Neonatal infection	1	0
Intracranial haemorrhage	3	0
Anoxia caused by placental disease	2	1
Toxaemia	2	0
Anoxia caused by labour	6	0
Total	41	6

294 days as the lower limit of gestation for post-term pregnancy

Perinatal mortality according to duration of pregnancy

Infants delivered post-term were subject to a significant increase in perinatal death. The perinatal mortality rate was 0 7% in term delivery and 2.2% in post-term delivery (Table I). Post-term pregnancy lost three times as many infants as term pregnancy The difference was statistically significant.

Causes of perinatal deaths

Nine separate causes of death were coded. Perinatal mortality at term and post-term, due to each of these causes, was studied. These causes include anoxia, birth injury congenital malformations, neonatal infection intracranial haemorrhage and toxaemia. Some deaths were from unknown causes (Table II).

Causes of perinatal death characteristic of post term delivery could not be found.

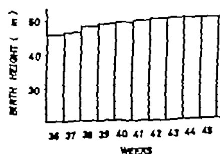


Fig 2 The mean birth height related to duration of pregnancy

Table VIII. *Forceps or vacuum extraction in post-term delivery*

	Pregnancies	Forceps or VE	%
Primipara			
Spontaneous labour	134	5	3 7
Induced labour	61	4	6 2
Multipara			
Spontaneous labour	61	1	1.6
Induced labour	19	1	3.3

in general, in agreement with other studies of this sort, but not with all. The reasons for this increased perinatal loss in the post-term group were analyzed as described, but no factors confined to the post-term group could be found. Ballantyne (1) suggested that the increased fetal mortality rate was due to the large size of the baby. Clifford (4), however states that the babies are not unduly large. This is in agreement with our findings.

It is accepted that an apparent increase in the perinatal mortality will be observed in post-term pregnancy but no particular cause has been demonstrated, and no treatment for this problem is yet available. A considerable amount of controversial material has been written about the effects of induced labour on fetal outcome in post term pregnancy and this is one of the most important problems in obstetrics. One school of thought as exemplified by Walker (21) and Hamilton (11) is rather dogmatic in suggesting that no patient should be permitted to go beyond 42 weeks of pregnancy if fetal loss is to be prevented, or unduly difficult labour is to be avoided whereas Browne (2), Eastman (8) and Daichman & Gold (5) are of the opinion that no special ill effects occur in the post-term patient, but rather that more trouble may well arise from the "routine induction of patients who have reached the end of the forty-second week. Perinatal mortality rates up to 4% and more have been reported by Gibberd (9) and other authors when such programme of induction has been instituted. At best, the weight of evidence indicates that induction of labour is not attended with any significant improvement in fetal outcome (13 20), and this is in agreement with our findings. The exception to this view is the report of McClure Browne (16) who describes an improved infant survival rate for patients past 42 / eeks gestation whose pregnancies were terminated by caesarean section. This is, in my opinion, a very interesting view of the management of post-term pregnancy

In the absence of a completely trustworthy method for routine termination of pregnancy at or after the forty-third week, the data presented here would be rather convincing argument for not recommending the elective interruption of pregnancy at the forty-third or even the forty fourth week. It appears certain that the time will soon be here when rapid and practical methods for determining placental function and fetal status in utero will be available permitting accurate selection of the small group of infants for whom further prolongation of intrauterine life would be hazardous, and allowing us to interrupt these high risk pregnancies by whatever method offers the least chance of harm to mother and her baby

It is the author's opinion that induction of labour solely because pregnancy has become prolonged, and in the absence of generally accepted obstetrical indications, would not appear to be justified by the data presented here.

ACKNOWLEDGEMENT

This study was carried out at the Department of Obstetrics and Gynaecology Osaka National Hospital. The author wishes to thank Dr T Ogura, Department Head of Gynaecology and Dr Y Kanabe, Department Head of Obstetrics, for their valuable suggestions. This paper was presented at the Twenty-Second Annual Meeting of Medical Affairs Bureau, Ministry of Health and Welfare on October 15 1967 at Sendai, Japan.

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Table IV *Forceps or vacuum extraction according to duration of pregnancy*

Weeks	Pregnancies	Forceps or VE	%
37–42	5 321	147	2.7
43 & over	275	11	4.0

prolonged. On the other hand, no increase in fetal distress as an indication for delivery was noted in post term delivery (Fig. 6)

Induction of labour

As previously mentioned, it is obvious that infants delivered post term were subject to an apparent increase in perinatal death. Although induction of labour is recommended when pregnancy has passed an arbitrary date there is evidence that "routine induction of labour at a predetermined post-term date is by no means a harmless procedure. We had no data regarding the specific indications for the induction of labour in this series, but labour was induced in 1 7% of all patients who delivered at term and 29 1% of all patients who delivered post-term. Those patients who delivered post term were divided into two groups: "spontaneous labour group" and "induced labour group" in order to compare the rate of perinatal death, prolonged labour caesarean section and forceps delivery or vacuum extraction in these two groups. No statistical difference could be demonstrated between the two groups on these criteria (Tables V VI, VII and VIII) It may be concluded that induction of labour does not lead to any significant improvement in fetal outcome.

Table V *Perinatal deaths in post-term pregnancy*

	Pregnancies	Perinatal death	
Primipara			
Spontaneous labour	134	2	1.5
Induced labour	61	2	3.3
Multipara			
Spontaneous labour	61	1	1.6
Induced labour	19	1	5.3

Table VI *Prolonged labour in post-term delivery*

	Pregnancies	Prolonged labour	%
Primipara			
Spontaneous labour	134	10	7.5
Induced labour	61	9	14.7
Multipara			
Spontaneous labour	61	4	6.6
Induced labour	19	2	10.5

Table VII. *Caesarean section in post-term pregnancy*

	Pregnancies	C.-section	
Primipara			
Spontaneous labour	134	8	6.0
Induced labour	61	4	6 4
Multipara			
Spontaneous labour	61	2	3.2
Induced labour	19	2	10.5

DISCUSSION

In our study group, a clear-cut increase was observed in the perinatal mortality rate as pregnancy became prolonged. The post-term mortality rate was about triple the death rate at term. This is,

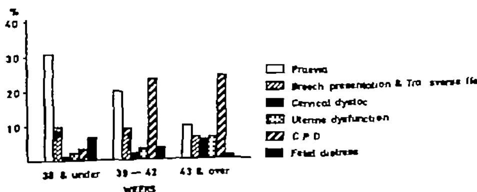


Fig 6 Indications for primary caesarean section.

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BEAD CHAIN URETHROCYSTOGRAPHY IN THE INVESTIGATION OF POSTOPERATIVE URINARY STRESS INCONTINENCE

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Abstract. The results of bead chain urethrocystography on 74 patients with postoperative urinary stress incontinence (USI) are reported. USI occurred after vaginal plastic operation, vaginal hysterectomy or suprapubic surgery performed for repair of uterovaginal prolapse and or USI. The findings in the bead chain urethrocystographic examination varied depending on the type of operation. Distribution by type (Green) based on loss of the posterior urethrovesical (PUV) angle and the angle of the urethral inclination is not suitable for every case of postoperative USI, for 20% of the cases displayed normal PUV angle during straining. The so-called base plate (Hutch) on the floor of the bladder did not remain flat during straining in any of the patients examined. In the planning of treatment for patients with postoperative USI attention should be paid not only to the urethral inclination and PUV angle but also to the anterior urethrovesical (AUV) angle and the position of the UV junction at rest and during straining.

Urinary stress incontinence (USI) in women is generally caused by an anatomic change, due to pregnancy that is localized to the floor of the pelvis (4). The nature of the anatomic injury causing USI has not been clarified conclusively. The so-called base plate theory introduced by Hutch (10) offers a logical explanation. "It describes a structure located in the base of the bladder which completely surrounds the internal urethral orifice and which is called the base plate. The base plate is the internal sphincter and to function properly it must be flat. Lateral cystograms show that in normal humans (both males and females) the natural position of the base plate is flat. In stress incontinence, however the base of the bladder falls into the space between the symphysis in the front and the anterior vaginal wall behind, and the base plate is forced into a funnel position by lack of space. The theory has attracted considerable attention although its significance still awaits final evaluation.

Various urethrocystographic methods have been employed for studying patients with USI. Their aim has been to clarify the relation between the urethra and bladder and the position of these structures vis-à-vis the surrounding tissues. Bead chain urethrocystography evolved by Stevens & Smith (13) and reviewed by Hodgkinson & Doud (9) has been widely used during the last two decades. The method is easy to perform and enables clear visualization of the urethra and the base of the bladder. It has been noted that the bead chain does not interfere with the free movement of the urethra and bladder when the patient is straining.

The straightening of the posterior urethrovesical angle (PUV) demonstrated in a lateral exposure made during straining has been regarded as significant in analysing roentgenologic changes typical of USI (5, 11). Equal attention has not been paid to the anterior urethrovesical angle (AUV). However, Unnérus et al. (14), Ball (2), Hutch (10) and Barnett (3) among other authors, have stressed the importance of the AUV angle for urinary continence. Hodgkinson (8) does not consider angle sizes expressed in degrees to be important, but is of the opinion that descent of the urethrovesical (UV) junction during straining to the lowest level of bladder is characteristic of USI.

Simplifying the distribution presented by Bailey (1), Green (6) classified USI patients into two types on the basis of lateral urethrocystographic examination performed during straining. Both

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Table I. *PUV angle in lateral bead chain urethrocystograms during straining after different types of operation*

Size of PUV angle	Operation: Vaginal plastic	Vaginal hysterectomy	Suprapubic	Total
Normal	6	6	3	15
Intermediate	12	6	1	19
Completely or nearly completely lost	28	10	2	40
Total	46	22	6	74

roentgenograms was determined in relation to the horizontal plane at the level of the inferior border of the symphysis. The vertical descent of the UV junction during straining was measured to an accuracy of ±5 mm.

RESULTS

Forty-six of the patients had had a vaginal plastic operation, 22 vaginal hysterectomy and six a suprapubic operation.

The distribution of the patients according to the size of the PUV angle (Table I) shows that the PUV angle was normal (less than 110°) after vaginal plastic surgery in six of the 46 cases, after vaginal hysterectomy in six of the 22 cases and after suprapubic operation in three of the six cases. Overall, fifteen patients (20%) had a normal PUV angle.

The urethral inclination (Table II) was normal (less than 45°) after vaginal plastic operation as well as following vaginal hysterectomy in only

Table II. *Urethral inclination in lateral bead chain urethro-cystograms during straining after different types of operation*

Urethral inclination	Operation: Vaginal plastic	Vaginal hysterectomy	Suprapubic	Total
Less than 45° (type I)	10	4	3	17
45° or more (type II)	36	18	3	57
Total	46	22	6	74

Table III. *Distribution by Green types after different types of operation. Includes cases with a PUV angle of more than 150°*

Green type	Operation: Vaginal plastic	Vaginal hysterectomy	Suprapubic	Total
I	6	2	1	9
II	22	8	1	31
Total	28	10	2	40

approximately 20% and after suprapubic operation in three of the six cases.

The distribution according to the type classification introduced by Green is shown in Table III. It includes only the 40 cases in which the PUV angle was completely or nearly completely lost. If the "intermediate" cases, representing the preliminary phase of USI are included, 12 (20%) patients belonged to type I and 47 (80%) to type II.

The series was divided into two groups according to the descent of the UV junction during straining (Table IV) taking 20 mm as the dividing value. Marked descent (20 mm or more) occurred after vaginal plastic surgery in 29 of the 46 cases, after vaginal hysterectomy in 10 of the 22 cases and after suprapubic surgery in two of the six cases.

Using lateral urethrocystograms taken at rest and during straining the cases were divided into three groups (A, B and C) according to the position of the UV junction in relation to the horizontal plane at the inferior border of the symphysis (Table V).

In group A the UV junction was located both at rest and during straining below this level in about 20% of cases following vaginal plastic operation and suprapubic surgery and in about 40% after vaginal hysterectomy.

In group B the UV junction was above the reference level at rest but below it during straining in approximately 60% of the cases after both vaginal plastic operation and vaginal hysterectomy and in two of the six cases after suprapubic surgery.

The UV junction in group C both at rest and during straining was above the horizontal level.

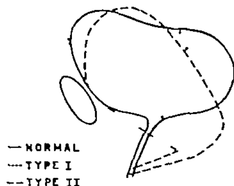


Fig 1 Schematic lateral representation of the urethra and bladder during straining in a normal case and in Green's types I and II

are characterized by complete or nearly complete loss of PUV angle. Urethral inclination in relation to the vertical plane is smaller than 45 in type I whereas it is 45 or more in type II (Fig. 1) According to Green, type I may be improved by vaginal surgery (Kelly stitches) Type II requires suprapubic surgery (e.g. the Marshall–Marchetti–Krantz operation) Following the above principles, Green (7) achieved a 91 per cent success rate in primary operations for USI.

It has been stated that the cystographic findings in postoperative USI differs from that in post partum USI (7 8) In the present work a study was made of the bead chain urethrocystographic findings in USI after gynaecologic operations of different types and of the suitability of the type distribution introduced by Green.

MATERIAL

The series consisted of 74 patients with postoperative USI operated on in 1965–1967 for uterovaginal prolapse and/or USI. The youngest patient was 36 and the oldest 71 years at the time of operation. The mean age was 5_1 years. Sixty-three patients had both pre and postoperative USI. Thirty-nine of them underwent vaginal plastic operations (Manchester operation and Kelly stitches), eighteen vaginal hysterectomy and six suprapubic surgery (Marshall–Marchetti–Krantz operation). Eleven of the patients had postoperative USI only Seven of them previously had the vaginal hysterectomy and four vaginal plastic surgery (Manchester operation). The diagnosis of USI was based on clinical examination and anamnestic data.

METHOD OF INVESTIGATION

Cystourethrographic examination was performed by TV fluoroscopy The contrast medium was 200 ml of micro-disperse barium sulphate solution (diluted and sterilized Mixobar®). The apparatus introduced by Pietila & Kauppila (12) was employed for the application of the bead chain and the instillation of the contrast medium. The aim of the bead chain was to reveal the shape and location of the urethra without the distortion which would be caused by for example a catheter The patient was erect during the examination and exposures were made in both the postero-anterior and lateral directions. They were made with the patient at rest and straining maximally The success of straining was checked fluoroscopically The same person performed all the investigations. No complications were observed.

In the examination of the urethrocystograms the PUV angle was determined from the lateral roentgenogram taken during straining (Fig. 2). An angle of less than 110° was regarded as normal while angles wider than 150° corresponded to Green term complete or nearly complete loss of PUV angle Both the cases in which the PUV angle was 110–150° and those in which it was normal (under 110°) but the length of the floor of the bladder just behind the urethra was less than 1 cm were considered to correspond to Green's "intermediate term.

The urethral angle of inclination was determined also from a lateral roentgenogram during straining. The angle is formed by the proximal urethra in relation to the vertical plane. Cases were classified into two groups whereby the limit value of 45 reported by Green was used as division.

The location of the urethrovesical junction in lateral

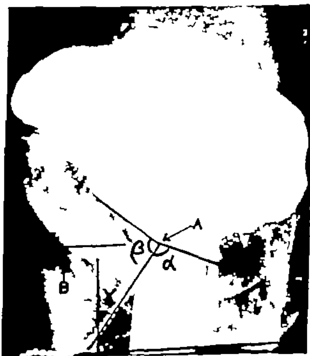


Fig The lines and points used and the angles measured in this study A, urethrovesical junction B horizontal line through the lower border of the symphysis, α posterior urethrovesical (PUV) angle β anterior urethrovesical (AUV) angle γ urethral inclination angle.

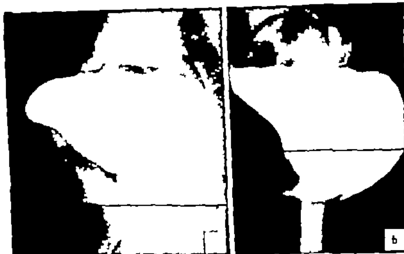


Fig. 4 Situation after vaginal plastic operation. The UV junction is above the level of the inferior border of the symphysis at rest (*a*) and below it during straining (*b*) (group B).

junction is situated at rest above the horizontal level of the inferior border of the symphysis and during straining below it. In addition, marked descent of the UV junction during straining is typical of this type of operation. It is possible that mobilization and elevation of the bladder neck was inadequate in these cases (Figs. 3 and 4). In some cases the posterior part of the bladder was incapable of following the descent of the UV junction during straining, resulting in loss of the PUV angle. A possible reason for this is that the floor of the bladder has remained fixed too high up after repair of the cystocele.

Green's group II appearances are also seen typically after *vaginal hysterectomy*. The UV junction is below the abovementioned horizontal level both at rest and during straining more frequently than after vaginal plastic surgery (Fig. 6). Elevation and fixation of the UV junction was inadequate in these cases. Considerable descent of the UV junction was observed in half of the cases.

A *supra pubic operation* was performed on six patients only and in three of them the appearances corresponded with Green's group II. In five of the cases the UV junction lay above the horizontal level of the inferior border of the sym-



Fig. 5 Situation after vaginal hysterectomy. The UV junction is below the level of the inferior border of the symphysis both at rest (*a*) and during straining (*b*) (group A).

Table IV *Descent of the urethrovesical junction in lateral bead chain urethrocystograms during straining after different types of operation*

Descent of UV junction	Operation: Vaginal plastic	Vaginal hyster ectomy	Supra-pubic	Total
Less than 20 mm	17	12	4	33
20 mm or more	29	10	2	41
Total	46	22	6	74

This occurred in about 15% of the cases after vaginal plastic operation and in three of the six cases after suprapubic surgery It was not observed after vaginal hysterectomy

Roentgenologic observations typical of groups A and B after vaginal plastic operation are shown in Figs. 3–4

Figs. 5–6 present the roentgenologic changes typical of groups A and B after vaginal hysterectomy The changes after suprapubic surgery and ascribed to groups B and C are shown in Figs. 7–8.

In 12 of the 15 cases in which the PUV angle was normal the UV junction during straining was at the lowest level of the bladder This was observed after vaginal plastic operation in all six patients and after vaginal hysterectomy in four of the six cases. There were three patients whose UV junction failed to descend to the lowest level of the bladder during straining. Two of these patients had undergone vaginal hysterectomy and one a suprapubic operation.

Table V *Position of the UV junction at rest and during straining in lateral bead-chain urethrocystograms after different types of operation. Groups according to the size of PUV angle*

Position of UV junction in relation to the horizontal level of the inferior border of the symphysis	Operation: Vaginal plastic	Vaginal hyster ectomy	Supra-pubic	Total
A. Below at rest and during straining	11	9	1	21
B Above at rest and below during straining	28	13	2	43
C. Above at rest and during straining	7	—	3	10
Total	46	22	6	74

Three patients displayed complete loss of the AUV angle in the course of straining (Fig. 8). They had had suprapubic surgery and their UV junction both at rest and during straining was above the horizontal level of the inferior border of the symphysis.

Not a single patient had a "base plate" that remained flat during straining, but in four cases the changes were small.

DISCUSSION

The results of an operative method with an unsatisfactory outcome (Table VI) show that roentgenologic changes following *vaginal plastic operations* tend to be in Green s group II and that the

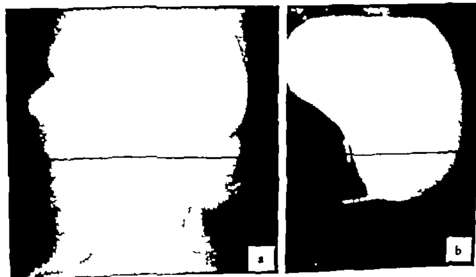


Fig 3 Situation after vaginal plastic operation. The UV junction is below the level of the inferior border of the symphysis both at rest ( ) and during straining (*b*) (group A).



Fig. 8. Situation after suprapubic operation. The UV junction is above the level of the inferior border of the symphysis both at rest (a) and during straining (b) (group C). N.B. loss of the AUV angle.

The "base plate" had either become funnel-shaped or bending of varying degree was observed in it before or behind the urethra.

To summarize, the present study suggests that in the examination of the bead chain cystograms of patients with postoperative USI attention should be paid not only to the urethral inclination and the PUV angle but also to the AUV angle and the position of the UV junction at rest and during straining. Observation of any changes regarding these makes individual rational therapy possible.

Table VI. *Trends of roentgenologic signs in lateral bead-chain urethrocystography according to the different types of operation*

equals 20 per cent, equals 10 per cent

	Operation		
	Vaginal plastic	Vaginal hysterectomy	Suprapubic
Loss of PUV angle			
Inclination of urethra 45° or more			
Green type I			
Green type II			
Descent of UV junction less than 20 mm			
Descent of UV junction 20 mm or more			
Position of UV junction in relation to the horizontal level of the inferior border of the symphysis			
A. rest below			
At rest above			
During straining below			
During straining above		–	

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Fig 6. Situation after v: nal hysterectomy The L junction is above the lev the inferior border of th symphysis at rest (*a*) an below it during straining (group B).

physis at rest and in three of them also during straining. Loss of the AUV angle was observed in these three instances, but the PUV angle was nor mal (Fig. 8). Fixation of the urethra and bladder neck to the pubic periosteum might have been too high or the fixation of the bladder too tight in these three cases. Two cases displayed notable (20 mm or more) descent of the UV junction. Operative fixation of the UV junction failed completely in these cases.

It is obvious from the present work that the typing introduced by Green and based on bead chain cystography is not directly applicable for postoperative USI as in one-fifth of the patients the PUV angle was found to be normal and in one-quarter it was the intermediate class.

The acute AUV angle was preserved in all e cept the three patients who had had a suprapul operation. As the PUV angle was normal in the cases the loss of the AUV angle was possibly factor leading to incontinence.

In five cases the UV junction failed to descen to the lowest level of the bladder during strain ing (two after vaginal hysterectomy and three af ter suprapubic operation) Hence this roentgeno logically demonstrable change too, is not suit able alone as a criterion for every case of post operative USI.

The theory best applicable to clarification of postoperative USI in the present study was Hutch's "base plate" theory A broken "base plate" was established in every one of these cases.



Fig 7 Situation after suprapubic operation The UV junction is abov the level f the inferior bo der of the symphysis at rest ( ) and below it during straining (*b*)

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CAESAREAN SECTION

A Ten-Year Study

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Abstract. Caesarean sections (C.S.) performed in the County of Kopparberg during 1951 1960 were studied. During this period 41 179 deliveries took place. The C.S. rate was 1 6%. In the Central County Hospital the rate was 2 7%, and in the County Hospitals 1.6%. Two maternal deaths occurred (0.3%). The perinatal mortality was 9 0%. In the Central County Hospital 25 of 436 infants were lost and in the County Hospitals 37 of 254. In the latter group the number of stillborn infants was high. In group of elective C.S. performed at term on healthy mothers only 1 of 163 infants died neonatally

In 1952 Belonoschkin & Malmnäs (1) presented study of the incidence of caesarean section (C.S.) in Sweden during three 5-year periods. During 1926–1930 the incidence was 0 25% during 1936–1940 0 47% and during 1946–1950 0 87%. Since then C.S. have been performed with increasing frequency especially in the recent years (2, 4) but ery few studies about C.S. in Sweden ha e been reported.

The present in estigation was performed in 1964 with the aim of studying the indications for and consequences of C.S. during the 1950s in a Swedish medical district. It is being published to elucidate the previous position of C.S. in Sweden and to give a background to a study of the last 5 years which is now planned.

MATERIAL

All C.S. performed in the County of Kopparberg during 1951 1960 were studied. During this period 41 179 deliveries took place (Table I). Group A included 431 C.S. performed at the Department of Obstetrics and Gynaecology of the Central County Hospital. Group B included 244 C.S. performed at the remaining County Hospitals. In these hospitals the obstetrical care was under the supervision of general surgeons. All Rh-immunized mothers are referred to centres outside the County with special facilities for exchange transfusions. These cases were therefore excluded from the study.

METHODS

The records for patients delivered by C.S. are re-examined with reference to indications for C.S., operative technique, operative and postoperative complications, anaesthetic methods, fetal asphyxia, birth weight and perinatal mortality. Only one indication was recorded for each case. If there were more than one indication, only the most relevant one was recorded. Previous classical C.S. myomectomy or Strassmann's operation were listed as scar in the uterine corpus. Indications such as multipara without living child" long-standing infertility previous complicated delivery were called "bad obstetrical history". Abnormal fetal presentations were sub-divided into three groups: 1) breech presentation in elderly primipara, 2) transverse lie and 3) other malpresentations. Breech presentation was always combined with other indications such as elderly primigravida, uterine inertia, cephalopelvic disproportion, diabetes or imminent fetal asphyxia. These cases ere recorded under the other indication rather than as "breech presentation" alone.

Lower segment transverse incisions as ell as classical incisions were used. In some cases the lower segment transverse incision was combined by an incision in the midline of the corpus, so-called inverted T-shaped incision. For special indications subtotal hysterectomies ere performed. In some cases sterilization was carried out by resection of the Fallopian tubes.

Atropine as used as routine pre-medication. However, some of the patients received morphine and scopolamine.

During the latter half of the period under investigation anaesthetists were available for the cases in Group A. All other cases were anaesthetized by an obstetrician, surgeon or nurse.

The following 5 types of anaesthesia ere used.

I. Diethyl-ether on open mask.

II. Local anaesthesia in the abdominal wall + diethyl-ether and diethyl-ether on open mask.

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ith diabetes mellitus was found dead in her bed uring the night between the 5th and 6th post perative day. On the previous evening she had een quite well with a normal blood sugar level. No definite explanation for her death was found t autopsy. In Group B one patient with placenta ccreta died from severe haemorrhage during peration.

Thrombosis was diagnosed 6 times in each group and pulmonary embolism once in Group A and twice in Group B. In Group A acute colitis was diagnosed twice. One of these patients was severely ill, but recovered. In general the most common postoperative complication was puerperal fever which occurred 37 times in Group A and 35 times in Group B. In all 79 patients or 18.3% had postoperative complications in Group A and 50 patients or 20.5% in Group B.

The use of antibiotic prophylaxis differed in the two groups. In Group A 59 of 352 patients (17%) without postoperative complications received antibiotic prophylaxis. In Group B 147 of 194 patients (76%) were given such therapy.

In Group A patients with living infants at birth received anaesthesia type I 22 times, type II 260 times, type III 128 times and type IV 19 times. In Group B the corresponding figures were: type I 128 times, type II 31 times, type III 45 times, type IV 2 times and type V 18 times.

Asphyxia at birth was diagnosed in 60 infants following 429 C.S. (14%) in Group A and in 43 of 224 (19%) in Group B. In these cases 11 and 10 infants respectively died neonatally. In Group A 12 infants who were not asphyxiated at birth died during the neonatal period. The corresponding figure in Group B was 7.

The cases with perinatal mortality were studied in regard to the indications for C.S. (*Table III*). It was found that those indications which involved increased risk for the fetus were often associated with perinatal deaths.

The effect of different types of anaesthesia on the fetal well being was studied on 163 electiv C.S. made on healthy mothers at term (Table IV). The lowest frequency of fetal asphyxia was noted in connection with anaesthesia Type II. The difference between this type and the other types of anaesthesia was probably significant ($p < 0.5$). In one case following anaesthesia Type III the infant died within 7 days of birth. The mother was one of those who by mistake got morphine as

Table II. *Indications for Caesarean Sections in the County of Kopparberg during the years 1951–1960*

Group A comprises the Central County Hospital and Group B the County Hospitals

	Group A	Group B
Cephalo-pelvic disproportion	177	43
Placenta praevia	44	47
Diabetes mellitus	43	0
Scar in the uterine corpus	30	7
Old primigravida	25	24
Bad obstetrical history	18	4
Breech presentation in elderly primi-gravida	15	7
Transverse lie	14	20
Other malpresentations	14	4
Toxaemia	11	38
Obstructed birth canal	10	3
Uterine inertia	9	7
Imminent fetal asphyxia	7	8
Hypertension	4	0
Abruptio placentae	3	18
Prolapsed umbilical cord	2	0
Imminent uterine rupture	1	6
Miscellaneous	4	8

pre-medication. The frequency of asphyxiated infants was 5.5% and the neonatal mortality 0.6% in these 163 elective C.S.

In 113 cases C.S. was performed for mechanical reasons within 24 hours of onset of labour. Ten of the infants were asphyxiated at birth but all survived the neonatal period. In 85 similar cases labour had lasted for 24 hours. At birth 17 infants were asphyxiated and 4 of them died within 7 days.

The causes of neonatal infant mortality is given in Table V. The most common causes were respiratory difficulties and immaturity. One infant died from severe anaemia, caused by rupture of an umbilical vessel in case of velamentous insertion. Only 2 infants died from congenital malformations. One died after an operation for atresia of the oesophagus.

The questionnaire was sent to 587 women and 512 (87%) answered. After lower segment incisions 136 women had been pregnant 167 times. These resulted in 49 vaginal deliveries, 92 C.S. and 26 spontaneous abortions. After a classical incision 75 pregnancies occurred in 62 women. These resulted in 39 vaginal deliveries, 28 repeat C.S., 2 laparotomies for uterine rupture, 4 spontaneous and 2 legal abortions. A dehiscence of the old uterine scar was seen once at repeat C.S. af

Table I *Deliveries and Caesarean Sections in the County of Kopparberg during the years 1951–1960*

	Deliveries (n)	Caesarean sections	
		n	%
Central County Hospital	16 044	431	2.7
County Hospitals	15 149	244	1.6
Cottage Hospitals	8 533		
Home deliveries	1 453		
	41 179	675	1 6

III. Ultra short-acting barbiturate + N_2O + O + succinylcholine + endo-tracheal intubation and intermittent positive pressure ventilation.

IV Cyclopropane + d-Tubocurarine + endotracheal intubation and intermittent positive pressure ventilation.

V Spinal anaesthesia.

In some cases anaesthesia type II was used until the delivery of the fetus and the placenta and then type III. These cases were recorded under the type used until delivery

Maternal post-operative complications could not always be classified as the records were often inadequate. Body temperature of more than 37 0°C in the morning of more than 37.5°C in the afternoon after the third post operative day in cases treated with antibiotics was recorded as puerperal fever if no other explanation was available in the record. I that way some cases of puer peral mastitis were probably recorded as post-operative complications. Treatment with antibiotics from the day of operation without any record of elevated body temperature or other signs of infection was considered as prophylactic.

The perinatal mortality was defined as the number of stillborn infants with crown-heel length of more than 35 cm and the numbe of infants who died within 7 days of birth. The Apgar scoring system was used only in the more recent years in G oup A. The degree of f tal asphyxia was hard to judge from the records. Thus it was only noted if the infant was asphyxiated or not. According to the nomenclature used during the 1950s infants with birth weight of 2 500 g or less were classified as premature.

I 1964 a questionnaire was sent out to all patients delivered by C.S. excluding those who were sterilized. In the questionnaire they were asked about further conceptions and the outcome of ll the subsequent pregnancies.

RESULTS

The C.S rate was 2 7% in Group A and 1 6% in Group B At the same time the C.S. rate for the whole County was 1 6% (Table I) Of the women attending the antenatal service of the Central County Hospital the C.S rate during 1956–1960 was 1.5% During the period under investigation the C.S rate was fairly constant. A total number of 611 women had 675 operations 549 women had one operation, 60 women two and 2 women three. Two maternal deaths occurred, one in each group.

The number of infants was 690 In Group A triplets were seen once and twins three times. In Group B twins occurred 10 times. The perinatal mortality was 9 0% (5 2% for mature and 43% for premature infants)

In Group A 436 infants were born, 401 of these were mature and 35 premature. Before the C.S 2 of the mature infants were dead and 10 died within 7 days of birth. There were no stillborn premature infants but 13 of the premature infants died neonatally Thus, the perinatal mortality at C.S in this group was 5 7% (3 0% for mature and 37% for premature infants) The average perinatal mortality at the Central County Hospital during the period under investigation was 3 3% (1 6% for mature and 35% for premature infants)

In Group B 254 infants were born. The number of mature infants was 219 14 of these were stillborn and 6 died during the neonatal period Six of the stillborn infants were thought to be alive when the C.S. was started. Of 35 premature infants 6 were stillborn and 11 died neonatally Only one of these stillborn infants was thought to be alive when the C.S was started. The perinatal mortality in this group was 14 6% (9 1% for mature and 49% for premature infants) The average perinatal mortality for the County Hospitals which contributed to the present investigation was 3 0% (1 9% for mature and 25% for premature infants)

Cephalo-pelvic disproportions was by far the most common indication in Group A (Table II) In Group B this indication was almost as common as placenta praevia and toxaemia The indications will be further discussed below

Lower segment incisions were made 414 times (96%) in Group A and 12 times in Group B Classical incisions were only made 6 times in Group A, while this type of incision was made 230 times (94%) in Group B Subtotal hysterectomies were performed 5 times in Group A and 2 times in Group B.

The most serious maternal complications were the two maternal deaths. In Group A one mother

than that reported by Belonoschkin and Malmnäs (1) during 1946–1950 and also lower than that of Group B. The most obvious difference between Group A and Group B was the large number of stillborn infants in Group B.

Differences were noted between Group A and Group B concerning the indications. The incidence of C.S. performed on mechanical and fetal indications was higher in Group A than in Group B. Toxaemia and abruptio placentae were less frequent as indication for C.S. in Group A than in Group B. This was due to an intentionally conservative approach to the treatment of these conditions at the Central County Hospital.

Many patients with complications during pregnancy with known narrow pelves and with previous complicated deliveries were referred to the Central County Hospital. This fact might to some extent explain the differences between the two groups. Other dissimilarities might be explained by different attitudes towards obstetrical problems between obstetricians and general surgeons. However one thing must be born in mind. The circumstances under which deliveries took place in the Central County Hospital and in the County Hospitals varied. The Central County Hospital had a delivery ward with a separate staff and good facilities for blood transfusions and intensive care. The County Hospital had a smaller staff common to the delivery and the postnatal wards and the facilities for blood transfusions and intensive care were limited. The conditions for a conservative expectant obstetric care were much better at the Central County Hospital. Problems arising on a County Hospital delivery ward had to be solved with regard to the limited facilities. Therefore C.S. was probably in some cases at least the best solution, even when the fetus was already dead.

In the present investigation as has been proved earlier (6, 7) the classical uterine incision proved to increase the risk of uterine rupture in a subsequent pregnancy. This operative technique was used in the County Hospitals because it was easier to perform and easier to teach the junior surgeons.

In the group of 163 elective C.S. performed on healthy mothers at term only one infant was lost. In this case the unfortunate use of morphine as pre-medication might have contributed to the fetal outcome. It might be concluded that the risk

Table VI. *The number of Caesarean Sections per 10 000 deliveries in Sweden during three 5-years periods (Belonoschkin & Malmnäs, 1952) and in the County of Kopparberg 1951–1960 (present investigation)*

	Belonoschkin & Malmnäs			Present investigation
	1926–1930	1936–1940	1946–1950	1951–1960
Cephalo-pelvic disproportion	7.6	13.0	22.0	52.6
Diabetes mellitus	0.04	0.04	1.1	10.3
Scar in the uterine corpus	0.1	0.3	0.9	8.9
Placenta praevia	5.2	11.6	15.8	21.8
Transverse lie	0.4	1.0	3.5	8.1
Breech presentation in elderly primigravida	0.04	0.3	1.2	5.3
Old primigravida	0.4	1.7	8	11.7
Imminent fetal asphyxia	0.1	0.2	0.7	3.6
Obstructed birth canal	0.5	0.5	0.8	3.2
Abruptio placentae	1.5	3.5	3.5	5.0
Imminent uterine rupture	0.3	0.5	1.4	1.7
Prolapsed umbilical cord	0.2	0.1	0.3	0.5
Pulmonary disease	0.04	0.15	0.2	0.2
Toxaemia	4.8	8.8	11.8	11.7
Heart disease	0.4	0.4	0.7	0.5
Uterine inertia	0.6	0.8	5.2	3.8

for the fetus to be delivered by C.S. was very small when the mother was healthy and the pregnancy at term.

The type of anaesthesia most suitable for C.S. has been a matter of discussion for a long time (3). In comparison between the different forms of anaesthesia the presence of asphyxia at birth was used as a criterion of safety. With this rough criterion there was some evidence that for the fetal safety local anaesthesia in the abdominal wall + divinyl- and diethyl-ether on open mask was the best method.

Prophylactic treatment with antibiotics was used more than 4 times as often in Group B than in Group A. In spite of this fact the frequency of postoperative infectious complications was similar in the two groups.

The large number of patients who were anxious about the possibility of another C.S. was remarkable. In most of these patients emergency operations had been performed. They were probably not mentally prepared for the C.S. It is also possible that communication between the doctor

Table III. *Perinatal mortality and fetal birth weight in relation to the indications for Caesarean Secti: the County of Kopparberg during the years 1951–1960*

Group A comprises the Central County Hospital and Group B the County Hospitals

	Birth weight 2 500 g or less				Birth weight above 2 500 g			
	Group A		Group B		Group A		Group B	
	Still-born	Neonatal death	Still-born	Neonatal death	Still-born	Neonatal death	Still-born	Neonatal death
Placenta praevia		5	1	5		2	2	2
Diabetes mellitus		1				5		
Toxaemia		4	1	3			1	
Abruptio placentae			3	1	1		6	1
Malpresentation		1				1		2
Scar in the uterine corpus		1			1	1		
Cephalo-pelvic disproportion							3	1
Imminent fetal asphyxia				1		1	1	
Cancer of uterine cervix		1						
Old primigravida				1				
Hydramnios							1	
Ileus			1					

ter previous lower segment incision and three times after previous classical incision.

Complaints of infertility were noted in 19 women and 94 women declared themselves to have a fear of another C.S. This fear had caused 21 women to avoid further pregnancies.

DISCUSSION

The increased frequency of C.S. in Sweden demonstrated by Belonoschkin and Malmnäs (1) was associated with a decrease in the maternal death rate from 9.5% in 1926–1930 to 26% in 1946–1950. In the present investigation the C.S. rate had increased about two times as compared to the rate in 1946–1950 while the maternal death rate was reduced to about one tenth.

In order to facilitate a comparison between the investigation of Belonoschkin and Malmnäs (1) and the present one, the number of C.S. made on different indications per 10 000 deliveries were calculated in each group (Table VI). An increase was noted for most of the indications, especially for cephalo-pelvic disproportion. The increase of diabetes mellitus as an indication for C.S. was probably due to a new approach to this problem during the 1950s (5) but a real increase of pregnancy in diabetic women might also have occurred. Abnormal fetal presentation and complications involving an increased danger for the fetus also showed a higher frequency as indications for C.S. On the other hand C.S. was not performed more often in cases of toxaemia in the present investigation. Uterine inertia as an indication for C.S. increased considerably during the last period of the investigation of Belonoschkin and Malmnäs but in the present investigation it was less frequent. This might have been due to an increased use of oxytocin.

In Group A the perinatal mortality was lower

Table IV. *Frequency of neonatal asphyxia at elective Caesarean Sections (C.S.) in relation to different types of anaesthesia (defined under Methods)*

Anaesthesia (type)	C.S.	Neonatal asphyxia
I	15	1
II	87	2
III	50	5
IV	11	1

Table V. *Causes of neonatal infant mortality at Caesarean Sections in the County of Kopparberg 1951–1960*

Respiratory difficulties	21
Immaturity	12
Cerebral haemorrhage	2
Suprarenal gland haemorrhage	1
Bleeding	1
Diabetic embryopathy	1
Hydrocephalus	1
Oesophageal atresia	1

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OVARIAN TUMOURS AND PEUTZ-JEGHERS SYNDROME

A Case of "Sex Cord Tumour with Annular Tubules" (Scully)

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Abstract Ovarian tumours (or tumour-like conditions) and Peutz-Jeghers syndrome are associated more frequently than by mere chance. This seems to be particularly true for the "Sex cord tumour with annular tubules" a new entity described by Scully in 1970. A case of this ovarian tumour found bilaterally together with unilateral mucinous cystadenoma in a patient with Peutz-Jeghers syndrome presented with discussion of the implications of this association.

The Peutz–Jeghers syndrome (PJS) is a hereditary disease characterized by grey-blue or brown pigmented spots on the oral mucosa and lips and benign polyps resembling hamartomas in the gastrointestinal tract, mostly in the jejunum, but occasionally also located in the nasopharyngeal, vesical and bronchial mucosa. The disease most often manifests itself during the first and second decade by abdominal colic due to intussusception or by anemia secondary to bleeding polyps.

Since Peutz in 1921 (5) described the syndrome and Jeghers et al. (4) in 1949 published a review several hundred cases have been published. The disorder may be considered unusual, but not extremely rare.

In 1957 Dormandy (1) stated that "ovarian tumours and cysts were found in a surprisingly large number of cases of PJS". This observation was later supported by Humphries et al. (3) and by Dozois et al. (2). The ovarian tumours described in the literature have been of various types, but in 1970 Scully (6) has emphasized a distinctive kind of ovarian tumour as being related to PJS with particular frequency. This he called "Sex cord tumour with annular tubules" (SCAT) describing 13 cases, 6 of which were associated with PJS. However due to incomplete investigation regarding PJS in the remaining 7 cases, this figure must be considered as minimal.

A case of PJS and ovarian changes, considered identical with Scully's SCAT is presented below.

CASE HISTORY

A 20-year-old unmarried woman with no family history of PJS was admitted in August 1970 because of a fast-growing abdominal tumour. She had menstruated regularly since her 14th year, but for 3 months prior to her admission to hospital her menstrual bleeding had been scanty. At this time there were no abdominal symptoms.

She was a normal-looking young woman with brown hair and grey eyes, well-developed breasts and normal axillary and pubic hair. Palpation of the abdomen revealed a large rounded mass stretching from the symphysis to the umbilicus. Rectal examination revealed a small normal uterus displaced to the right by a large cystic mass.

Laboratory tests: Hemoglobin 13.1 g%, ESR 4 mm/hour. Urine analysis: no glucose or protein.

Subsequent laparotomy disclosed a left-sided ovarian cyst with a smooth surface, approximately 13 cm in diameter. The right ovary was slightly enlarged with a smooth surface and a thickened fibrous capsule. The left-sided cyst was removed and a wedge biopsy was taken from the right ovary. There were no postoperative complications.

A urine analysis 2 months after the operation showed normal output of total estrogens, pregnanediol, 17-ketosteroids and 17-ketogenic steroids.

The patient was readmitted in December 1970 because of abdominal colic and vomiting. Now a spotted brownish pigmentation was observed on the lips and the oral mucosa, and the possibility of PJS was discussed.

Laparotomy revealed an intussusception of the small intestine caused by a polyp 3 cm in diameter and located 90 cm distally to the duodenum. 150 cm of the jejunum was devitalized and was resected together with the polyp. No changes, other than those described previously were found in the right ovary. The postoperative course was

and the patient was not satisfactory More thorough information about the conditions which made the operation necessary would perhaps have reduced maternal anxiety for the future.

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Abstract Ovarian tumours (or tumour-like conditions) and Peutz-Jeghers syndrome are associated more frequently than by mere chance. This seems to be particularly true for the "Sex cord tumour with annular tubules" new entity described by Scully in 1970. A case of this ovarian tumour found bilaterally together with unilateral mucinous cystadenoma in patient with Peutz-Jeghers syndrome is presented with discussion of the implications of this association.

The Peutz–Jeghers syndrome (PJS) is a hereditary disease characterized by grey-blue or brown pigmented spots on the oral mucosa and lips and benign polyps resembling hamartomas in the gastrointestinal tract, mostly in the jejunum, but occasionally also located in the nasopharyngeal, vesical and bronchial mucosa. The disease most often manifests itself during the first and second decade by abdominal colic due to intussusception or by anemia secondary to bleeding polyps.

Since Peutz in 1921 (5) described the syndrome and Jeghers et al (4) in 1949 published a review several hundred cases have been published. The disorder may be considered unusual, but not extremely rare

In 1957 Dormandy (1) stated that ovarian tumours and cysts were found in surprisingly large number of cases of PJS" This observation was later supported by Humphries et al. (3) and by Dozois et al (2) The ovarian tumours described in the literature ha been of various types, but in 1970 Scully (6) has emphasized a distinctive kind of ovarian tumour as being related to PJS with particular frequency This he called "Sex cord tumour with annular tubules (SCAT) describing 13 cases, 6 of which were associated with PJS. However due to incomplete investigation regarding PJS in the remaining 7 cases, this figure must be considered as minimal.

A case of PJS and ovarian changes, considered identical with Scully's SCAT is presented below

CASE HISTORY

A 20-year-old unmarried woman with no family history of PJS was admitted in August 1970 because of fast-growing abdominal tumour She had menstruated regularly since her 14th year, but for 3 months prior to her admission to hospital her menstrual bleeding had been scanty At this time there were no abdominal symptoms.

She as normal-looking young woman with brown hair and grey eyes, well-developed breasts and normal axillary and pubic hair Palpation of the abdomen revealed a large rounded mass stretching from the symphysis to the umbilicus. Rectal examination revealed small normal uterus displaced to the right by large cystic mass.

Laboratory tests: Hemoglobin 13.1 g% ESR 4 mm/hour Urine analyses: no glucose or protein.

Subsequent laparotomy disclosed left-sided ovarian cyst with smooth surface, approximately 13 cm in diameter. The right ovary was slightly enlarged ith smooth surface and thickened fibrous capsule. The left-sided cyst was removed and edge biopsy was taken from the right ovary There were no postoperative complications.

A urine analysis months after the operation showed normal output of total estrogens, pregnanediol, 17-ketosteroids and 17-ketogenic steroids.

The patient was readmitted in December 1970 because of abdominal colic and vomiting. Now spotted brownish pigmentation was observed on the lips and the oral mucosa, and the possibility of PJS as discussed.

Laparotomy revealed an intussusception of the small intestine caused by polyp 3 cm in diameter and located 90 cm distally to the duodenum. 150 cm of the jejunum was devitalized and was resected together with the polyp. No changes, other than those described previously were found in the right ovary The postoperative course was

and the patient was not satisfactory More thorough information about the conditions which made the operation necessary would perhaps have reduced maternal anxiety for the future

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Fig. 3. Adjacent to the annular tubules high vacuolated stroma cells are seen forming solid and cord-like masses (ca. 100).

ant literature in order to find a connection between PJS and ovarian tumours.

It is not easy to evaluate how often the association between PJS and ovarian tumours occurs. In the survey from 1966 Humphries et al. (5) compiled 180 cases of PJS from the literature, 9 of which had ovarian tumours, i.e. a frequency of 5%. In a survey published in 1970 by Dozois et al. (2) the percentage however is higher. It comprises 115 cases of PJS, sufficiently documented to ascertain the presence or absence of ovarian tumours (including the 9 cases from the above-mentioned material). Among these 115 cases, 18 ovarian tumours were found in 16 patients with primary histological diagnosis as follows: 5 granulosa cell tumours, 1 Brenner tumour, 1 dysgerminoma, 5 cystadenomas, 4 non-neoplastic cysts and 2 with uncertain diagnosis. Some of these diagnoses were later questioned by Scully (6) who among the granulosa cell tumours found 3 cases of his SCAT.

The most interesting point about the association between PJS and ovarian tumours lies in Scully's discovery mentioned in the introduction. In his own files and in those of the Massachusetts General Hospital he found 10 cases of the SCAT and added 3 more found among previously published cases of PJS with ovarian tumours. Among these 13 cases of SCAT 6 were known to be associated with PJS. In the remaining 7 no signs of PJS were described, but no accurate relevant information was available. Some of these patients might have had undiscovered mucosal spots or symptomless intestinal polyps.

As Scully writes, and the present case confirms, an ovarian biopsy is necessary to rule out a sex cord tumour because the tumour can be very small. Therefore it still remains an open issue

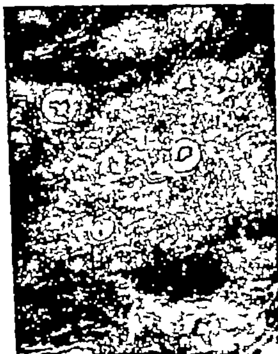


Fig. 4. Many foci of tubular elements in the right ovary (ca. 30).

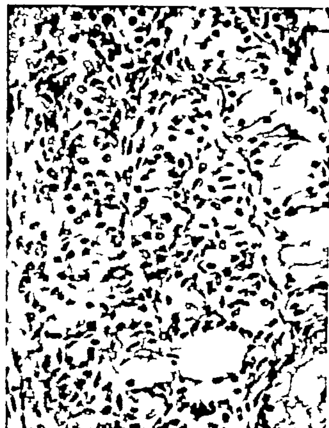


Fig 1 Transition from tubular cells to Leydig-like cells (ca. ×250).

uneventful and at a follow-up visit in April 1971 she was in good condition. There were no dyspeptic complaints or menstrual irregularities.

Histopathological examination

Left sided ovarian cyst. The specimen shows typical mucinous cystadenoma with a slightly folded membrane lining the cyst wall from which smaller secondary glandular formations extend into the wall, which consists mainly of connective tissue. Moreover some cortex-like ovarian stroma is preserved, and in this there are many foci of simple or complex annular and tubular structures. These elements are lying singly as well as in small groups and are made up of epithelial cells in palisaded or radial arrangement mostly with two rows of nuclei, one towards the periphery and one towards the center In many of the tubules small, central, predominantly homogenous PAS-positive precipitations are seen, but only in one section is small focal calcification present. I some areas of the specimen, closely connected with the tubules the stroma contains cells with homogeneous light, eosinophili or vacuolated cytoplasm, forming diffuse or cord-lik masses. Some of these intertubular cells morphologically resemble Leydig cells although no crystalloids of Reinke are present, but others seem to be related t the large vacuolated tubule-forming cells from which, in some areas, they cannot be distinguished (Fig. 1).

Right ovary This biopsy consists mainly of ovarian cortex, forming rather broad border of dense cellularity

Many annular and tubular formations like those in the left ovary are present, irregularly distributed, and here the majority of them form complex structures (Figs. and 4). The hyaline central precipitations may be slightly lamellar or vacuolated. Large masses of vacuolated, apparently lipid-containing cells are found in the stroma, mostly in close relationship to the tubular structures, from which they seem to emerge directly (Fig. 3). The structure in these cell-masses is solid, but with a somewhat cord-like or tubule-mimicking pattern. Intertubular cells like those described in the left ovary are also found in this specimen. Germ cells hav not been found in connectio with the described lesions.

The intestinal polyp The polyp from the small intestine is partly necrotic but in better preserved areas well differentiated mucosal glands are found deep in the muscular wall consistent with the hamartomatous intestinal tumours found in PJS.

Histopathological diagnoses. Mucinous cystadenoma of the left ovary "Se cord tumour with annular tubules in the wall of the left ovarian cystaden ma and in the right ovary Hamartomatous polyp of the small intestine.

DISCUSSION

The present case is that of a classical Peutz Jeghers syndrome with mucocutaneous pigmentations and a hamartomatous polyp in the small intestine but with no family history As in several of the former published cases the patient had been treated for an ovarian tumour before the PJS was diagnosed. Not until she had an acute intestinal obstruction, due to intussusception, were the typical pigmentations of the oral mucosa observed. The case gave rise to a review of the rele



Fig 2 Tubule with central precipitations of PAS-positiv material. The nuclei f the epithelial cells are seen predominantly in tw rows, one towards the periphery and one towards the centre (ca. ×150).



Fig 3 Adjacent to the annular tubules light vacuolated stroma cells are seen forming solid and cord-like masses (ca. 100).

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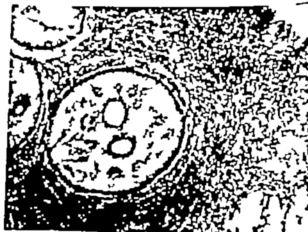


Fig. 2. Tubule with central precipitations of PAS-positive material. The nuclei of the epithelial cells are seen predominantly in two rows, one towards the periphery and one towards the centre (ca. × 150).

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THE VALUE OF RENOGRAPHY IN THE ASSESSMENT OF OBSTRUCTION IN THE UPPER URINARY TRACT

An Investigation of 45 Cases of Carcinoma of the Cervix Uteri Treated by Wertheim Hysterectomy

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Abstract. Simultaneous renography and intravenous urography has been done to assess the value of renography in detecting the presence of upper urinary tract obstruction. 45 women were treated by Wertheim operation (radical hysterectomy and pelvic lymph node dissection) and were investigated preoperatively and at 14 days, 4–6 months and 1, 2 and 3 years postoperatively. A total of 193 investigations have been done. Good agreement was found in 33 cases, i.e. 73% (21 had normal flow and 12 had varying degrees of obstruction). A false diagnosis of obstruction was made in 8 cases (i.e. 17%) after renography and in 3 cases after urography. Renography would appear to be a reliable screening method for detecting ureteric obstruction after Wertheim operation. A normal renogram excludes obstruction. Abnormal renograms should be followed by urography not only because the high sensitivity of renography can lead to false diagnosis of obstruction, but also to detect the site of the lesion.

A considerable number of investigations, based on postmortem material, have shown that the commonest cause of death following carcinoma of the cervix uteri is, apart from the disease itself, uraemia due to ureteric stenosis. The frequency of the latter complication in different series was as high as 80% (Altvater & Imholz, 1960; Böckler, 1961; Brunschwig & Frick, 1956; Corbie, 195 ; Gansau, 1960; Henriksen, 1949; Käser & Iklé, 1961; Kirchhoff, 1960; Muth, 1957; Pahl, 1958; Pearson, 1936; Pockrandt, 1961; Remé & Altvater, 1961) and 30–60% of these patients died of uraemia (Brunschwig & Frick, 1956; Buchmann, 1956; Corbie, 1952; Gansau, 1960; Henriksen, 1949; Käser & Iklé, 1961; Pearson, 1936; Sotto et al., 1960).

Following these results, a large number of workers have investigated the frequency of urological complications in apparently cured patients, and have shown that severe changes, arising from the results of therapy, are not at all uncommon. The incidence of uraemic death following ureteric stenosis in patients with cured cervical cancer varies in the different series from 3–12% (Altvater & Imholz, 1960; Böckler & Prinz, 1959; Gansau, 1960; Hartl, 1961; Krantz, 1960; Käser & Iklé, 1961; Sotto et al., 1960). If one ignores postmortem material and looks only at patients with apparently cured cervical cancer who have had intravenous urography after treatment then one finds that the frequency of uni- or bilateral changes lies in the range of 7–18% (Burns et al., 1960; Gansau, 1960; Kirchhoff, 1960; König, 1962; Muth, 1957; Pockrandt, 1961); this, of course, includes not only those with minimal changes but also those severe enough to require surgical intervention.

It was not possible to separate the effects of operation and of radiotherapy: singly or in combination they cause, sooner or later, damage to the urinary tract (Hartl, 1961). Benson & Hinman (1955) found that ureteric damage was most common after surgery; Gansau (1960) Mauch & Halsorff (1964) after radiotherapy; whilst Berta et al. (1963) were equally emphatic that late damage to the ureters occurred most frequently after combined treatment. Though advances in both operative and radiotherapeutic techniques have reduced the incidence of fistulas, the un-

how frequently these changes in the ovaries can be detected in women with *PJS* and whether there is any connection between them and the occurrence of polyps in the intestine.

Scully describes his tumour as varying from microscopic size to 17 cm in diameter with a soft or firm consistency and with calcification in some cases. The colour is often yellowish and in two of the cases bilateral involvement was evident, but the contralateral ovary was not microscopically examined in more than 4 cases. As to the detailed microscopical appearance of the tumour I refer to Scully's original work (6) and the description of the present case. As here, 4 of the tumours in Scully's material were found to have many foci in the ovarian stroma.

In this short presentation it is not appropriate to discuss the histopathogenesis of the Scully tumour in detail, but some of the problems involved include Is it a real neoplasm or is it better regarded as a hamartoma or maldevelopment? From which cells does it originate? In this connection it may be of interest to note the close topographical and morphological relationship between the tubular cells proper and at least some of the intertubular cells found in our case.

Scully is of the opinion that the tumour originates from the granulosa cells, but grows in a pattern which is characteristic of the Sertoli cells. He therefore chooses to call it "Sex cord tumour with annular tubules" However he does at the same time express doubt from which cellular elements the tubules originate, and it is also left open whether the large intertubular cells are to be considered as Leydig or lutein cells. He believes that the tumour is a neoplasm and not a hamartoma. Small lesions such as those found in our case are difficult to regard as a real neoplastic tumour but here observations of a single case cannot be decisive Scully's opinion is based on the fact that lesions of all sizes up to large tumour masses have been observed in other cases.

The detection of endometrial hyperplasia and menstrual irregularities in a number of women with ovarian tumours and PJS has led to the suspicion that the tumour may secrete estrogen. Our patient was menstruating regularly and a urinary assay of estrogens was normal, which does not suggest any increased estrogen production in this case.

In conclusion it is recommended that all women with the PJS should be carefully examined to exclude the presence of an ovarian tumour and conversely if SCAT is diagnosed, the possibility of PJS must be considered, leading to an inspection of the oral mucosa and to a complete examination of the intestinal tract.

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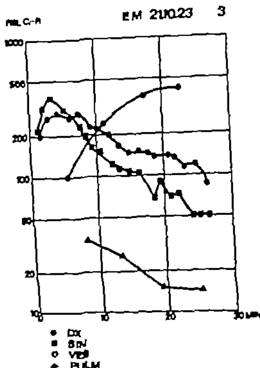


Fig. 1. Normal urography: renography: moderately impaired outflow on the right side; left side normal (14 days post-operatively).

counted over one of the lungs, not only as comparison with the renal counts, but also to measure the fall in blood level of radio-iodinated hippuran which is used as the test substance (radio-iodine labelled ortho-iodo-hippuran). If the renal counting shows depression of urinary outflow the patient is allowed to get up and move around for five minutes and the investigation then continues for further ten minutes. Initially the renogram studies were done according to Mårtensson and Vikterlöf (1965) method of using conventional scintigram apparatus with 3 inch sodium iodide crystal (T 1) and 10 cm diameter conical collimator; the indicator was 10 microcuries ¹³¹I-labelled hippuran. In order to increase the precision of the investigation the technique was modified at the end of 1966. A 5 cm diameter cylindrical collimator replaced the earlier one; an injection of 10 microcuries ¹³¹I-hippuran was used to localize the bladder and kidneys, the investigation was then done with 15 microcuries ¹³¹I-labelled hippuran. The detector counts for 20 seconds over each renal area and takes 10–15 seconds to move from one side to the other in this way about twenty measurements are made on each side and recorded on a penrecorder. A further 4–5 counts are made over one lung and the bladder. The renograms are assessed qualitatively; simple quantitative evaluation of possible reductions in renal function and outflow characteristics are done without going into extensive numerical calculations since these have been considered both unnecessary and unsuitable for our purpose (cf. Jockes & Sherwood 1967).

MATERIAL

During the period 1965–1969 seventyseven patients in the Gynaecological Department, Karlstad, have undergone Wertheim's operation for carcinoma of the cervix Stages I–II A (Stages II B–IV have received only radiotherapy). Preoperative assessment has included palpation under anaesthesia, cystoscopy, blood and urine biochemistry, heart and lung x-rays, intravenous urography and in 43 patients renography. Postoperatively the patients have remained catheterized for 14 days—longer, if the residual urine measurements were greater than 50 ml. A minimum daily urine volume of 1 200 ml is maintained during the first two postoperative weeks.

Postoperatively 45 of the 77 patients have undergone urography and renography at the following intervals: 14 days, 2–6 months, 1, 2 years or more frequently if the results showed progressive outflow obstruction. The other patients were followed with intravenous urography alone because the isotope department was closed part of the year. There was no selection of patients in respect to whether combined urography-renography or only urography has been done; 16 of the 45 patients have also had radiotherapy (10 postoperatively, 5 preoperatively and 1 both pre- and postoperatively).

ber of ureteric strictures has not decreased to the same extent. Medina et al. (1960) however consider when the prognosis is taken into account, that fistula formation is not the most serious complication strictures have a worse prognosis because the associated renal changes can be clinically quiet" unless routine assessment is undertaken at regular intervals after treatment has been completed.

The question arises whether the absence of parametrial infiltration which is generally agreed to be a causative factor in stricture formation, could give a pointer to the appearence of ureteric obstruction Various authors have said that this is not always so (Buchmann 1956 Pahl, 1958 Mönch & Haltorff 1964 Kremling et al. 1962) Thus Mönch & Haltorff (1964) found that 87 of 225 women given radiotherapy for cervical cancer in whom intravenous urography was done, showed contracted scarring, thickening or infiltration of the parametria 46 of these showed no hindrance to urinary flow

Regular assessment of the urinary tract to detect outflow obstruction is as important as checking for the appearance of recurrent cancer in which the urological examination also plays a large part. Half of the cases of ureteric stenosis with hydronephrosis and reduction in renal function can be symptom-free unless infection supervenes. This applies particularly to those with unilateral obstruction but also to the bilateral cases even up to the insidiously developing final stages of uraemia. The only possibility of detecting and treating ureteric stenosis at an early stage is to do regular urological check-ups in all cases of cervical cancer both pre- and postoperatively Urinary outflow changes can appear within 4–8 weeks postoperatively after radiotherapy they can take 1–3 years to appear (Fochem & Grünberger 1954 Busse & Muth, 1957) A large proportion of the obstructions found in the early postoperative period, with a varying degree of hydronephrosis, resolve spontaneously within a year or so (Malik, 1960 Kolstad, 1969) others remain and may progress. Separation of these two groups can only be achieved by regular reassessment so that those with progressive obstruction can be treated before irreparable renal parenchymal damage occurs. Hohenfellner & Janisch (1964) for example in their well-managed series of 533 Wertheim operations found that one of the reasons for a reduction in the incidence of severe ureteric and renal damage was the careful postoperative control of urinary tract function and consequent early therapy

The commonest method for examining urinary flow from the kidney is intravenous urography In 1956 Taplin et al. introduced isotope renography i.e. the study of urine secretion and flow by the measurement of radioactivity over the kidneys following the injection of a suitably isotope labelled indicator Experience has shown that the method has its greatest use in the detection of obstruction to urinary flow (Berghard et al., 1967 Cosbie Ross et al. 1963 Garnett, 1961 Gerbie & Flanagan 1962 Joekes & Sherwood, 1967 zum Winkel et al. 1961). In contrast to intravenous urography it gives no anatomical information but only demonstrates the presence of obstruction which may lie at any level in the affected ureter On the other hand, it does not disturb normal physiology is simple and safe for the patient, is relatively cheap and gives a much smaller radiation dose than intravenous urography Renography can be done at much shorter intervals and can be repeated if the results are technically unsatisfactory On account of its high sensitivity it is of greater value as a negative rather than a positive test: a normal renogram indicates that renal function and urine flow are unimpaired and that further investigation by other methods is unnecessary (Aurell, 1971) It should not be regarded as a replacement for intravenous urography renal angiography or the standard tests of renal function but as a complement to these and above all, as a screening method to detect those patients in whom wider assessment is desirable (Berghard et al 1967 Brites Patricio & Baptista, 1963 Cosbie Ross et al. 1963 Dische et al. 1963 Falk et al 1969 Green et al. 1965 Joekes & Sherwood, 1967 Kiviniitty 1966 Mårtensson & Vikterlöf 1965 Roddick et al. 1964).

METHODS

We have used the technique earlier described by Mårtensson & Vikterlöf (1965). The patient, in a normally hydrated state, lies prone whilst single scintillation counter alternately measures the radioactivity over each kidney An advantage with this procedure is that the detector and measurements geometry are the same for each side, thus giving firmer basis for making quantitative comparisons between the two sides. We have also



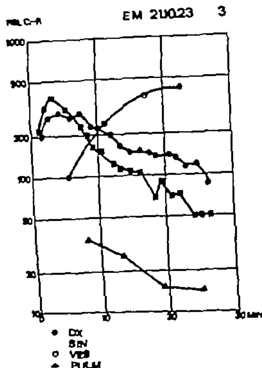


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Postoperatively 45 of the 77 patients have undergone urography and renography at the following intervals: 14 days, 2–6 months, 1–2 years or more frequently if the results showed progressive outflow obstruction. The other patients were followed with intravenous urography alone because the isotope department was closed part of the year. There was no selection of patients in respect to whether combined urography-renography or only urography has been done: 16 of the 45 patients have also had radiotherapy (10 postoperatively, 5 preoperatively and 1 both pre- and postoperatively).

ber of ureteric strictures has not decreased to the same extent. Medina et al. (1960) however consider when the prognosis is taken into account that fistula formation is not the most serious complication strictures have a worse prognosis because the associated renal changes can be clinically "quiet" unless routine assessment is undertaken at regular intervals after treatment has been completed.

The question arises whether the absence of parametrial infiltration, which is generally agreed to be a causative factor in stricture formation could give a pointer to the appearence of ureteric obstruction. Various authors have said that this is not always so (Buchmann 1956 Pahl, 1958 Mönch & Haltorff 1964 Kremling et al. 1962) Thus Mönch & Haltorff (1964) found that 87 of 225 women given radiotherapy for cervical cancer in whom intravenous urography was done showed contracted scarring, thickening or infiltration of the parametria 46 of these showed no hindrance to urinary flow

Regular assessment of the urinary tract to detect outflow obstruction is as important as checking for the appearance of recurrent cancer in which the urological examination also plays a large part. Half of the cases of ureteric stenosis with hydronephrosis and reduction in renal function can be symptom-free unless infection supervenes. This applies particularly to those with unilateral obstruction, but also to the bilateral cases even up to the insidiously developing final stages of uraemia The only possibility of detecting and treating ureteric stenosis at an early stage is to do regular urological check ups in all cases of cervical cancer both pre- and postoperatively Urinary outflow changes can appear within 4–8 weeks postoperatively after radiotherapy they can take 1–3 years to appear (Fochem & Grünberger 1954 Busse & Muth, 1957) A large proportion of the obstructions found in the early postoperative period, with a varying degree of hydronephrosis, resolve spontaneously within a year or so (Malik, 1960 Kolstad, 1969) others remain and may progress. Separation of these two groups can only be achieved by regular reassessment so that those with progressive obstruction can be treated before irreparable renal parenchymal damage occurs. Hohenfellner & Janisch (1964) for example in their well-managed series of 533 Wertheim operations found that one of the reasons for a reduction in the incidence of severe ureteric and renal damage was the careful postoperative control of urinary tract function and consequent early therapy

The commonest method for examining urinary flow from the kidney is intravenous urography In 1956 Taplin et al. introduced isotope renography i.e. the study of urine secretion and flow by the measurement of radioactivity over the kidneys following the injection of a suitably isotope labelled indicator Experience has shown that the method has its greatest use in the detection of obstruction to urinary flow (Berghard et al 1967 Cosbie Ross et al. 1963 Garnett, 1964 Gerbie & Flanagan 1962 Joekes & Sherwood, 1967 zum Winkel et al. 1961) In contrast to intravenous urography it gives no anatomical information but only demonstrates the presence of obstruction which may lie at any level in the affected ureter On the other hand, it does not disturb normal physiology is simple and safe for the patient, is relatively cheap and gives a much smaller radiation dose than intravenous urography Renography can be done at much shorter intervals and can be repeated if the results are technically unsatisfactory On account of its high sensitivity it is of greater value as a negative rather than a positive test. a normal renogram indicates that renal function and urine flow are unimpaired and that further investigation by other methods is unnecessary (Aurell, 1971) It should not be regarded as a replacement for intravenous urography renal angiography or the standard tests of renal function but as a complement to these and above all, as a screening method to detect those patients in whom wider assessment is desirable (Berghard et al. 1967 Brites Patricio & Baptista, 1968 Cosbie Ross et al. 1963 Dische et al. 1963 Falk et al. 1969 Green et al. 1965 Joekes & Sherwood, 1967 Kiviniitty 1966 Mårtensson & Vikterlöf 1965 Roddick et al. 1964)

METHODS

We have used the technique earlier described by Mårtensson & Vikterlöf (1965). The patient, in a normally hydrated state lies prone whilst single scintillation counter alternately measures the radioactivity over each kidney An advantage with this procedure is that the detector and measurements geometry are the same for each side thus giving firmer basis for making quantitative comparisons between the two sides. We have also

CASE HISTORY

A 43-year-old nulliparous woman ith cervical cancer stage II A. Preoperative radiotherapy: intrauterine and intravaginal radium (4 938 mgh) and external high-voltage therapy (Co^{60} 3 000 r).

A Wertheim's operation as done one month after completion of the radiotherapy. Preoperative renogram and urogram were normal.

14 days postoperatively normal urography: renography however, demonstrated moderately impaired outflow on the right side; left side normal (Fig. 1)

Two months postoperatively urography showed the presence of distally located ureteric stricture on the right side ith moderate ureteric and pelvic dilatation there; renography: obvious deterioration of outflow on the right side. The left side was again normal (Fig. 2).

A percutaneous pyelostomy (Molin & Ulmsten, 1971) was done on the right side because of worsening hydronephrosis. An ureterectomy as done at subsequent date. When last seen (August 1971) the right kidney as found to be still functionally impaired.

CONCLUSION

This survey emphasizes the importance of preoperative and postoperative assessment of flow conditions in the upper urinary tract after radical surgery for carcinoma of the cervix uteri. Ureteric obstructions are seen to appear early in the postoperative period, the majority disappear spontaneously within a few months whilst some will orsen Increasing ureteric dilatation and hydronephrosis need surgical treatment before renal function deteriorates. The isotope renogram has proved to be an excellent screening method for the detection of changes in urinary outflow from the kidney It is less taxing, gives a smaller radiation dose and permits more frequent assessment than intravenous urography A normal renogram excludes ureteric obstruction an abnormal renogram should be supplemented by intravenous urography to localize the lesion

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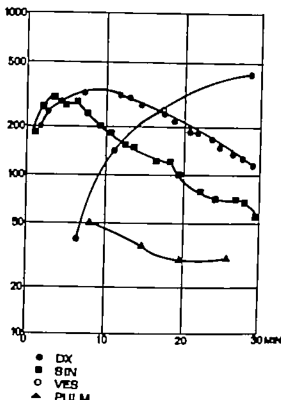


Fig 2 Urography distal ureteric stricture on the right side with moderate ureteric and pelvic dilatation above renography obvious deterioration of outflow on the right side (2 months post-operatively).

223 urographies and 11 renographies have been done. Comparisons between the two methods have been possible on 193 occasions: the time-interval in the other investigations has been longer than one week. 26 patients have been assessed over a period of 3 years, the reminder for two years after their operation.

RESULTS

In 21 patients both renographic and urographic studies have shown normal outflow tract condition.

In 8 patients with normal urograms, the renograms have on occasion demonstrated a slowing of urinary flow on one or both sides. This has later become normal in all cases. In five of these patients the changes were seen in the renograms done at 14 days apart from one patient, they were normal again at 2 months. Two cases showed changes first at the 2 months test and in one of these the changes were present for a year One patient showed changes only at a 6 months assessment.

In 12 patients renography indicated delay in urinary outflow from one or both kidneys whilst urography demonstrated the presence of pelvic and ureteric dilatation Eight of these patients had only moderate changes which reverted to normal, or almost so. Three patients showed progressive changes which necessitated reimplantation of one ureter into the bladder this was followed by normal reno- and urograms. In one patient ureteric obstruction was diagnosed one year after her Wertheim operation and was found to be due to the presence of a stone which was removed. Of the others, nine showed changes at the 14 day test whilst two of them were detected at 2 months (the 14 day test had not been done).

Three patients have shown temporary unilateral ureteric dilatation at urography whilst the renogram was normal.

One patient was found to have a slow urinary outflow on one side at renography done 14 days postoperatively the urogram was normal. The 2 months assessment showed that the obstruction had progressed with abnormalities evident on both the renogram and the intravenous urogram (see the following case history)

CASE HISTORY

A 41-year-old multiparous woman with cervical cancer stage II A. Preoperative radiotherapy: intrauterine and intravaginal radium (4 938 mgh) and external high-voltage therapy (Co^{60} 3 000 r).

A Wertheim operation was done one month after completion of the radiotherapy. Preoperative renogram and urogram were normal.

14 days postoperatively: normal urography; renography however, demonstrated moderately impaired outflow on the right side; left side normal (Fig. 1).

Two months postoperatively: urography showed the presence of distally located ureteric stricture on the right side with moderate ureteric and pelvic dilatation. Renography: obvious deterioration of outflow on the right side. The left side was again normal (Fig. 2).

A percutaneous pyelostomy (Molin & Ulmsten, 1971) was done on the right side because of worsening hydronephrosis. An ureteroneocystostomy was done at a subsequent date. When last seen (August 1971) the right kidney was found to be still functionally impaired.

CONCLUSION

This survey emphasizes the importance of preoperative and postoperative assessment of flow conditions in the upper urinary tract after radical surgery for carcinoma of the cervix uteri. Ureteric obstructions are seen to appear early in the post operative period, the majority disappear spontaneously within a few months whilst some will worsen. Increasing ureteric dilatation and hydronephrosis need surgical treatment before renal function deteriorates. The isotope renogram has proved to be an excellent screening method for the detection of changes in urinary outflow from the kidney. It is less taxing, gives a smaller radiation dose and permits more frequent assessment than intravenous urography. A normal renogram excludes ureteric obstruction, an abnormal renogram should be supplemented by intravenous urography to localize the lesion.

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URINARY TRACT INFECTIONS IN ASSOCIATION WITH RADIUM THERAPY FOR GYNECOLOGICAL CANCER

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Abstract. The purpose of the study was to investigate the influence of radium therapy on the development of urinary tract infections in patients with gynecological carcinoma. The series comprised 167 patients treated with one or more radium applications The patients' mean age was 57 years The treatments were given at 14-day intervals by modified Stockholm method. Before each radium application, quantitative bacterial cultures of the urine was carried out. The patients' urological status was observed throughout the period of treatment. About 16%, had urinary tract infection on admission. Even after the first radium treatment the number of urinary tract infections had trebled, and despite prophylactic chemotherapy it remained at the same high level until the completion of therapy Radium therapy and the accompanying manipulations produced severe bladder irritation often associated with infection It is obviously necessary to devote increasing attention to the occurrence of latent urinary tract infections in the treatment of patients with gynecological cancer By so doing, unnecessary tissue irritation, hydronephrosis, and later fistula formation can be reduced

Complications in the urinary tract are often found in connection with the treatment of gynecological cancer (1 7 11 12, 20 22, 23 24). Widholm et al (27 31) have shown that about 30% of the women admitted for treatment of genital cancer have an elevated urea N or creatinine and about 40% have hydronephrotic changes even before therapy is started. According to Kirchoff (8) the frequency of urological complications in cured cases of cervical cancer is not less than 20–25%

Kottmeier & Gray (12) demonstrated a direct correlation between the dosage levels in bladder and rectum, and the injury rate. The limit for a high injury rate to the bladder was a dose of more than 6000 gamma or more than 9000 total Whelton (29) studied cervical infections in cases of cervical carcinoma and showed that inflammation may spread after radium therapy The literature has generally discussed genital infections following Ra therapy and tissue reactions of later origin, whereas information on the incidence of urinary tract infections in the different phases of radium therapy is scant. Hittmair (5) Kümper & Penning (14) and Kümper (13) have shown that the presence of inflammation in the bladder mucosa increases the irritating effect of irradiation on the tissue and therefore contributes towards the development of ulceration. On the basis of his series of Wertheim operations, Timonen (25) has shown that the presence of urinary tract infection during the course of radiation therapy definitely increases the rate of complications and also adversely affects the therapeutic results. On the basis of 188 cases of female genital carcinoma treated with radiation for the first time, Kümper (13) showed that significant bacteriuria developed in 50% On admission the rate of bacteriuria was 11 per 188 and after the first Ra application about 30% Adequate treatment of these infections helps to prevent the development of ulceration and irreversible tissue changes in the course of radiotherapy

The purpose of the present study was to investigate the effect of Radium therapy on the development of urinary tract infections and the associated changes in the urinary tract during the different stages of the treatment.

MATERIAL AND METHODS

The series comprised a total of 167 patients with gynecological carcinoma treated with one or more Ra applications. The distribution of the material was the following: cervical carcinoma Stage I, 48 patients, Stage II, 32,

Table I *Summary of the urinary culture findings before treatment and after the individual Ra applications*

The total series comprised 167 patients. All culture results after the different applications are included. Cultures with >100 000 bact./ml

	No. of pats.	%
Culture 1 (before treatment)	27/167	16
Culture 2 (after 1st Radium application)	49/126	38
Culture 3 (after 2nd Radium application)	31/73	42
Culture 4 (after 3rd Radium application)	32/77	41

Stage III, 44 corpus carcinoma, 24 and some other gynecological carcinoma, the remaining 19 patients. The patients mean age was 57 years. The Ra applications were carried out at 14-day intervals by a modified Stockholm method (10, 26).

The following tests were taken, if possible, before every Ra application. bacterial culture of midstream urine and typing of the bacteria, urine sediment microscopy urea-N or creatinine, haemoglobin, and X-ray intravenous pyelogram before and after treatment. Urine specimens were taken before Ra applications on the same day the first even before therapy was started. Quantitative bacterial culture was carried out in "Uricult tubes, from which sensitivity determination cultures were also taken (2, 3 4 11 12, 13 15). The samples were transported without delay to the laboratory in the same building where cultures were made immediately

Bacterial cultures with >100 000 bacteria/ml were classified as pathological (2, 6) The average duration f a radium pplication was about 15 hours, and during this time the patient had an indwelling catheter in the bladder and the vagina was packed. The total dosage of radiation was recorded on the basis of direct measurement from the bladder and rectum during treatment (26). On the first day of treatment, routine prophylactic chemotherapy was instituted, usually with sulphonamide preparation, and with few exceptions the drug was continued throughout the intervals between applications until the course of treatment was completed. Chemotherapy was changed during the course of treatment as indicated by the results of bacterial resistance tests.

The series is not homogeneous as regards the severity of disease and treatment. Many patients, after having only one pplication, underwent an operation or were transferred elsewhere for treatment.

Table I shows the urinary culture findings before treatment and after each application. Since from a therapeutic point of view the series was divided after the *first treatment* int those undergoing surgery and those continuing the radiation treatment, the numbers of patients per group are different.

Table I shows that bacteriuria was detected in 16% of the control specimens taken before the first Ra application. The number of bacteria is seen to increase after each Ra application despite prophylactic therapy After the 3rd application, 41% of the urine specimens were infected. The biggest group consisted of the patients receiving only one Ra application.

In Table II we have tried to analyse in some detail the results of bacterial cultures before and after the first application in a selected group where the tumour growth had not extended to the urinary tract.

Table II reveals that the number of urinary tract infections was more than doubled after the first Ra application. Bacteriuria due to the E. coli group increased by 50% and the number of cultures yielding Klebsiella bacteria was trebled.

In the next table w have tried to analyse the cases in which the patient was given three consecutive Ra applications at approximately two-week intervals, with simultaneous prophylactic chemotherapy

The table reveals that after the first application the number of cases with bacteriuria (>100 000/ml) in the group was trebled and, despite the treatment of urinary tract infection, remained all the time at this high level. It must be pointed out that this group represents advanced cases of carcinoma, cervical carcinoma Stage I, 3 patients, Stage II–III, 21 patients, and patients th vaginal and endometrial carcinomas. Only 10 of these patients are still living, and therefore it is impossible to draw any conclusions regarding the long-term effect of infection on the urinary tract. The measured dosage of radiation to the bladder in this group of 26 patients averaged 540 r. However in patients whose bacteriuria after the third application was >100 000/ml, the bladde dose was distinctly higher roughly 3 060 r The doses were measured from the bladder and rectum with gammameter in connection with Ra applications.

The bladder irritation produced by Ra therapy has also been estimated by observing the changes in urine sediment. It was found that the leucocyte and erythrocyte counts ere usually doubled after each application and remained at the elevated level throughout the period of Ra therapy

Table II *Bacterial determination before and after the 1st Ra application*

The group comprised 83 patients, who only had this one pplication

Culture	Bacteria >100 000/ml	
Culture I (before treatment)	14 patients (17 %)	
	E. coli	10
	Klebsiella	3
	gram pos. coccus	1
		14
Culture II (after 1st application)	32 patients (39 %)	
	E. coli	20
	Klebsiella	9
	gram pos. coccus	2
	staphyloc.	1

A total of 74 patients were examined by intravenous urography both before and after therapy. The interval from the first to second urography averaged 3 months, range 3 weeks to 36 weeks. Only two patients had any pathological findings on the first urography. Later follow-up examinations, however, revealed pathology in 16 patients, with uni- or bilateral hydronephrosis, without any distinct correlation to the earlier bacterial findings. Fourteen of these patients had cervical carcinoma Stage I-III, and two had carcinoma of both cervix and body of the uterus. No significant rise in the urea-N values could be shown during the treatment. The mean haemoglobin of the present patients on admission was 12.2 g/100 ml and after treatment 11.7 g/100 ml.

Only [illegible] patients gave [illegible] history on admission that they had had frequent urinary tract infections previously and 13 patients had [illegible] history of one previous episode of urinary tract infection.

DISCUSSION AND CONCLUSIONS

The above results reveal that some 16% of the patients admitted with genital cancer have a urinary tract infection with ≥ 100 000 bact./ml. This is about four times the frequency usually reported from mass examinations of this age group (4). Even after one Ra application the incidence of urinary tract infections is doubled, and after the second application it still increases, to almost treble, [illegible] trend similar to the findings reported by Kümper (13).

A comparison of the results of urine sediment microscopy with those of the bacterial cultures reveals that the percentages of both leucocytes and erythrocytes are approximately doubled, but the increases are not proportional to those in the number of bacteria. The changes in urine sediment are partly due to the Foley catheter and partly to Radium irritation and vaginal packing. According to Kass (7), the use of an indwelling catheter for 24 hours produces [illegible] urinary tract infection in 50% of the cases. In the present series, the mean duration of each Ra application was 15 hours. It may be taken, therefore, that the number of infections attributable to the indwelling catheter probably was less than this figure.

Table III showed that prophylactic sulphonamide or other antibacterial therapy did not inhibit urinary tract infection or reduce it during the third Ra [illegible]pplication. The unsuccessful result of this treatment is [illegible]t least partly explained by the fact that this group of patients represented advanced carcinoma.

The irradiation doses received in the region of

Table III. *The result of bacterial determination in 26 patients who had undergone three Ra applications*

Cultures	Bacteria > 100 000/ml	
Culture 1 (before treatment)	3 patients (12%)	
	E. coli	1
	Klebsiella	2
Culture 2 (after 1st Radium application)	9 patients (35 %)	
	E. coli	3
	Klebsiella	5
	gram pos. coccus	1
Culture 3 (after 2nd Radium application)	9 patients (35 %)	
	E. coli	5
	Klebsiella	4
Culture 4 (after 3rd Radium application)	9 patients (35 %)	
	E. coli	4
	Klebsiella	3
	gram pos. coccus	2

the ureters and bladder are often larger than has been assumed. Intraureteral crystal measurements have given readings differing by 10–20% from the calculated dosage (28 [illegible] 30).

The irritation of tissue by radiation apparently provides a favourable soil for a permanent infection (18 [illegible] 21). Bearing in mind that each Ra application is accompanied by about 15 hours of indwelling catheter and efficient packing of the vagina, it is evident that the urinary tract is infected in connection with such therapy *in nearly* 50% of the cases. When the application is repeated twice again at about 14-day intervals, or the patient is transferred to a surgical unit, the probability that a chronic urinary tract infection may develop is great. The routine sulphonamide nitrofurantoin prophylaxis we have used does not seem to be adequate therapy for these cases. Treatment must be individually chosen in each case on the basis of bacterial resistance tests, and it must continue throughout the period of treatment and even afterwards until the urine remains free of infection. Kümper (13) has shown that urinary tract infection during radiotherapy can be cured by specific chemotherapeutic drugs. If the patient has a urinary tract infection when external radiotherapy begins, the chances of developing hydronephrosis or even urinary tract fistulas are much greater than in cases without infection. The irritation of the radiotherapy easily obscures the symptoms of the presence of a urinary tract infection. For this reason, frequent quantitative bacterial cultures of the urine, both

in the course of and long after the therapy are of importance.

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LONG-TERM USE OF DEPOT MEDROXY PROGESTERONE ACETATE AS A CONTRACEPTIVE

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Abstract Depot Medroxy Progesterone Acetate 150 mg every 3 months was given to 231 women followed through 4 671 women/months. The same progestogen in 300 mg dosage was given as six-monthly injections to 92 women followed through 904 women/months. These two dosage schedules were 100% effective in fertility control, the crude drop out rate reached 33.7% and 51.0% respectively. The menstrual cycles were markedly disorganised. The re-establishment of normal menstrual and endometrial cycles and fertility after stopping therapy is reported. The effects on the genital organs, glucose tolerance, thyroid and liver function tests were studied.

An ideal contraceptive, one which is perfectly acceptable to all people, has yet to be discovered. The search is therefore still on for a safe inexpensive and effective method. The attractiveness of long-acting hormone preparations, has led to the investigation of several progestational agents in an injectable form as contraceptives.

As these injected contraceptives apparently seem an appropriate method for fertility control in developing countries, we carried on several extensive trials (1–4) in an attempt to obtain a suitable hormone preparation.

This communication deals with the long-term use of depot medroxy progesterone acetate in two dosage schedules as a three- and six-monthly contraceptive.

MATERIAL AND METHODS

This work was undertaken at the birth control clinic of Ain-Shams University between April 1968 and December 1970. The trial group included 323 women of proven fertility as indicated by two or more previous successful pregnancies. To achieve maximum fertility lactating women and those who had received previous hormonal contraceptives were not accepted in this trial. The age of the patients ranged between 19 and 41 years (average 30.8 ± 5.2 S.D.) and their parity ranged between two and 14 (average 4.6 ± 2.4 S.D.).

A complete history was taken from all cases who were subsequently subjected to a thorough clinical examination. The participants included two groups: Group I, composed of 231 cases who were given 150 mg depot medroxy progesterone acetate every 3 months. Group II, included 92 cases who were given 300 mg every 6 months. Injections were started on the 2nd to the 4th day of the cycle. They were given intramuscularly deep in the gluteal region.

All cases were followed up monthly during therapy and for variable periods (not less than 10 months after stopping therapy).

Vaginal smears were taken from all cases before starting therapy and repeated during treatment. Cervical mucus was studied from 80 cases during different months of therapy for quantity consistency elasticity ferning, sperm penetration and sometimes post-coital tests were performed.

Endometrial biopsies were taken from 80 cases and repeated in different phases of the cycle during and after stopping therapy: a total of 740 biopsies were studied. Intravenous glucose tolerance was performed in 40 cases before therapy and repeated one or two years after. Liver function tests, thymol and zinc turbidity albumen/globulin ratio and Bromsulphalein tests were performed in another 34 cases. Direct thyroid function tests including serum protein bound iodine (p.B.I.), free thyroxine (T_4) and radioactive iodine uptake (2 Hr and 24 Hr) were done in 21 cases receiving 150 mg medroxy progesterone.

Ovarian and tubal biopsies were examined from 20 cases who accepted tubal sterilization as a method of contraception. The specimens were obtained during different phases of menstrual cycles or periods of amenorrhoea. All these cases had received injections for 18 to 30 months.

At the end of the trial, 108 women had received 10 injections, lasting for 3 months, while 34 women had received 4 injections, lasting for 6 months.

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in the course of and long after the therapy are of importance.

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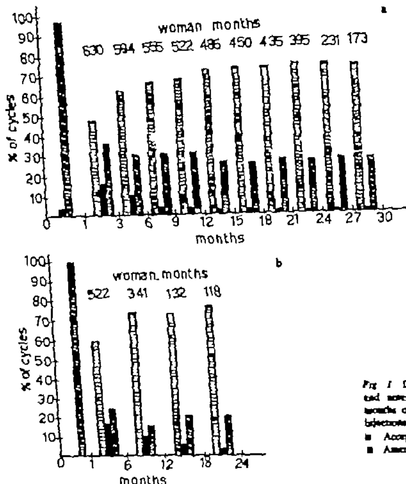


Fig 1 Incidence of acceptable, aberrant and amenorrhoeic cycles during different months of therapy (*a*) With three-monthly injections (*b*) With six-monthly injections.
■ Acceptable cycles; Aberrant cycles;
■ Amenorrhoeic cycles.

Within the first 2 months of therapy proliferative endometrium was found in 59% of cases in the theoretical second half-cycle, while remaining cases showed either mixed or insufficient secretory pattern. The secretory response was mainly stromal. From the 3rd to the 9th month, the endometrium was either proliferative or quiescent where the glands appear as simple tubular and inactive. From the 10th month onward, preatrophic or atrophic changes became apparent and their incidence increases with therapy. The detailed description of these changes was reported elsewhere (5).

The fallopian tubes showed marked changes; the mucosa appeared static and suppressed. A uniform mucosal pattern was obtained during different phases of menstrual cycles indicating the absence of cyclical changes that were usually detected in controls.

The epithelial cells were much smaller than those of controls in any phase of cycle, their cytoplasmic content was much reduced and more deeply stained. Their cilia were stunted and inconspicuous. The number of secretory cells and the amount of P.A.S. positive secretory material was markedly diminished in the isthmic and interstitial portions of the tube, while peg cells showed a marked increase. The vascularity of the mucosal folds and the muscle coat was markedly decreased.

Ovarian specimens examined during acceptable cycles revealed signs of follicular activity. All stages of follicular development and luteinised cystic follicles were encountered. Mature graafia

Table I *Reasons for leaving trial*

	Group I[a]	Group II[b]
I Drug-related causes	57	37
Amenorrhoea or scanty menses	41	24
Menorrhagia	8	9
Irregular cycles	6	4
Varicose veins	1	—
Vaginal discharge	1	—
II. Causes not related to drug	10	2
Changing residence or travel	6	1
Opposition of husband	2	—
Desire to conceive	1	1
Divorce	1	—
III. Non-relevant causes[c]	11	8
Total	78	47

[a] Received 150 mg every 3 months.
[b] Received 300 mg every 6 months.
[c] Our social workers failed to contact them.

RESULTS

Effectiveness

This progestogen proved 100% effective in fertility control in the two dosage schedules tried.

Acceptability and drop outs

The primary acceptability was rather good, but the crude drop out rate reached 33 7% in the group receiving three monthly injections, of these 58.9% discontinued during the first year of therapy The twenty cases who accepted tubal sterilization as a method of contraception were not included as drop outs. The crude drop out rate for six monthly injections was almost as high, 51 0% Causes of dropping out are listed in Table I. Many of the cases have received either 10 three-monthly or 4 six monthly injections. A total of 4 671 and 904 woman-months were studied with the 3 and 6 monthly injections respectively

Cycle control

It is rather difficult to present cycle control because the menstrual cycles were markedly disorganized. We adopted therefore a simple classification that takes into consideration the number of days of bleeding/month, the rhythm and the amount of bleeding. Cycles were therefore classified as amenorrhoeic, aberrant and acceptable. Amenorrhoeic cycles when no bleeding occurs in any given month (0 days bleeding/month) Aberrant cycles when there is bleeding of more than 8 days/month, excessive bleeding of any duration, interrupted or repeated bleeding or spotting in the same month. Acceptable cycles characterized by 1 to 7 days of consecutive moderate bleeding/month. The cycle control of 3 and 6 monthly injections during different periods of study are presented in Fig. 1 a and b respectively

It is apparent that the incidence of amenorrhoea was very high, ranging between 48.3% and 74.2% with the three monthly injections and between 59 6 and 77% with the six monthly injections. The incidence of amenorrhoea revealed a constant tendency to increase with therapy while aberrant bleeding revealed a tendency to decrease. A total of 1 452 bleeding cycles occurred with three monthly injections. The amount of bleeding was scanty in 30.4% and excessive in 18% of these studied cycles.

Side effects

No important side effects other than menstrual disorders were reported. Minor complaints as headache, dizziness, diminished libido vaginal discharge and nervousness occurred in a small percentage of the cases. One case developed marked varicose veins of the lower limbs and was advised to stop injections. No significant changes were reported in the blood pressure or body weight.

Intravenous glucose tolerance revealed slight elevation in few cases, but the variations were statistically insignificant. Likewise the direct thyroid and liver function tests revealed no significant changes.

Effect on reproductive system

The changes in cervical mucus become evident after the first month of therapy At the theoretical time of ovulation the mucus was thick and scanty its elasticity was markedly diminished. It revealed either minimal (Grade I) or no ferning. It was usually hostile to spermatozoa, although occasionally a good post-coital test was obtained.

The vaginal smear showed a marked anti-estrogenic effect in different phases of the cycle the pyknotic index ranged between 8 and 28. The smears did not reveal, however any criteria suggestive or conclusive of malignancy

activity was preserved, except in prolonged amenorrhoea.

As the re-establishment of fertility ,normal menstrual and endometrial patterns may be delayed after stopping therapy it is advised that the use of this progestogen be avoided in nulliparae or those with only one child. This method may be suitable for multiparae who accept unpredictable menstrual cycles and prolonged amenorrhoea for the sake of easy control of fertility by three- or six-monthly injections.

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follicles were found in two cases, they were, how ever deeply situated away from the surface of ovary A corpus luteum was detected in one case indicating ovulation The number of corpora albicantia was less than that of controls.

Ovarian tissue examined during amenorrhoea (11 cases) revealed either complete follicular suppression where only primordial follicles were detected or showed a polycystic pattern. Polycystic ovaries contained multiple nonluteinized follicular cysts of variable sizes. The available cortical tissue was reduced.

Re-establishment of normal menstrual and endometrial cycles and fertility

Ninety-eight cases who had received ten three monthly injections were followed after stopping therapy at the end of the trial. These include 83 cases whose husbands accepted the condom as a method of contraception and 15 cases who desired further pregnancy and did not adopt any contraceptive measure. The remaining cases who used I U C.D.s or pills were not included in the follow up. Seventy seven cases re-established a normal menstrual cycle within 2–6 months (average 4 1 ± 2.1 S.D) Repeated endometrial examination of 50 cases during the second half of the cycle revealed the occurrence of a good secretory pattern within 5 to 11 months (average 8.3 ± 2.8 S.D)

In 21 cases, amenorrhoea persisted for more than 6 months (7–12) They were given depot estrogen (Estradiol undecylate, Schering Berlin) 30 mg I.M. They usually responded by withdrawal bleeding within 15 days.

Among 15 cases who wished to conceive, six cases conceived after 8 to 13 months respectively the remaining nine cases had been followed up for 7 to 11 months.

Thirty-six cases of those given six-monthly injections were followed up, of which 28 cases re established normal menses within 3 to 7 months. One case who wished to conceive became pregnant 11 months after stopping the therapy

DISCUSSION

Depot Medroxy Progesterone Acetate in the two dosage schedules tried, proved highly effective in fertility control as a three or six-monthly contraceptive. It is worth mentioning that when 400 mg/cc was tried as a six-monthly contraceptive, the failure rate was high (6) This may have been the result of a high concentration of drug which may cause defective absorption from the site of injection. The high clinical efficacy of this progestogen has also been reported by several other workers (9–12)

The menstrual cycles were completely disorganized amenorrhoea or aberrant bleeding frequently occurred. The incidence of amenorrhoea showed a tendency to increase with therapy After one year it reached over 70% The administration of oral ethinyl estradiol 0.02 mg (progynon C, Schering Berlin) two tablets/day for 5 to 7 days was usually successful in inducing withdrawal bleeding in cases of amenorrhoea or in stopping aberrant bleeding.

Although the primary acceptability was rather good, the crude drop out rate reached 33 7% and 51 7% with the three- and six-monthly injections respectively This high drop-out rate (23 8% with three and 25% with six-monthly injections) was mainly the result of menstrual disorders and most of the cases discontinued therapy during the first year Many women feared that amenorrhoea might indicate pregnancy or they wrongly believed that amenorrhoea denoted retention of menstrual blood with a potential serious toxic effect on the body Spotting and irregular bleeding are troublesome as they compel our women to prohibit coitus due to religious habits. Excessive or prolonged bleeding on the other hand is alarming.

The drug did not produce significant changes in glucose tolerance, liver and thyroid function tests. The blood pressure and body weight did not vary significantly during the trial.

This progestogen controls fertility through many varied actions, including effects on cervical mucus, endometrium, fallopian tubes and ovaries. In most instances, the mucus became hostile to spermatozoa, while the endometrium appeared unsuitable for implantation. The tubal mucosa became static and markedly suppressed with a marked reduction in the number of secretory cells and the amount of P.A.S. positive material. These changes may render the tubal environment unsuitable for an ovum, spermatozoon or early fertilized egg. Ovarian tissue revealed an absence of ovulation in most instances, although follicular

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EXPERIENCE WITH DEPO-MEDROXYPROGESTERONE ACETATE (DEPO-PROVERA) AS A CONTRACEPTIVE AGENT

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Abstract. Pure, long-acting gestagen, medroxyprogesterone acetate (Depo-Provera), was tested as a contraceptive agent in a dose of 150 mg i.m. every 12th week. 139 women were observed for altogether 1 251 calendar months without any undesired pregnancy occurring in the group. The normal regular menstrual cycle was replaced initially by unpredictable vaginal bleeding and/or spotting, most often gradually followed by amenorrhoea. About 15% of the women discontinued the trial because of such menstrual irregularities. About 6% of the women stopped because of weight gain. Only few other side-effects occurred. Withdrawal of the preparation is followed by regular ovulation and menstruation, but often not until long after the last injection. The method therefore appears to be suitable mainly for women who have decided to have no more children.

The simplicity of the method, the absence of estrogen (probably involving a reduced risk of thromboembolic disease and effect on the liver), the high level of safety and reliability combined with good acceptability despite unpredictable bleeding, and the fact that the method is well tolerated by women who had previously been unable to tolerate hormonal contraceptives containing estrogen, suggest that the method should have a place in our contraceptive armamentarium. However it should not be recommended as the primary choice, in any case not for younger women. The method is probably very suitable for women who have tried other methods without success, followed by unplanned pregnancies and subsequent legal abortions.

Trials of hormonal contraception by the parenteral administration of longacting steroids were started at the beginning of the 1960s. Different types and doses of gestagenic steroids were tried alone and combined with oral or parenteral estrogen. In trials with injections of pure gestagen the best results were produced by long-acting medroxyprogesterone. A dose of 150 mg i.m. every 12th week was generally used. Since several investigations 10, 13, 15, 17, 19 in other countries have shown medroxyprogesterone in this dose to be a safe and well tolerated contraceptive, it was decided to try the method in Sweden.

MATERIAL

Medroxyprogesterone acetate (6-alpha-methyl-17-alpha-acetoxyprogesterone) is a synthetic 17-acetoxyprogesterone derivative with a strong gestagenic and anti-gonadotrophic effect.

In Sweden this long acting agent for i.m. use is marketed under the name of Depo-Provera® (Upjohn AB), not yet for sale as a contraceptive.

139 healthy women belonging to different socio-economic classes took part in the study. They are grouped according to age and parity in Tables I and II.

The study was carried out from September 1969 to April 1971 and covered 1 251 calendar months. The distribution of the total number of injections among the women is given in Table III.

At the beginning of the study the women were carefully informed about the advantages and disadvantages of the method and, in particular, about the irregular bleeding episodes to be expected. The women were given special menstrual calendars, on which they were instructed to note all the days on which they bled enough to require sanitary protection. They were also informed that the method offered roughly the same degree of protection as the combined type of oral contraceptive.

The first injection was usually given early in the cycle. Several women had their first injection within a few days of a legal abortion, and occasionally immediately post-partum. The women were advised not to expect any contraceptive effect until 14 days after the first injection. The same warning was also always given when the interval since the preceding injection exceeded 12 weeks.

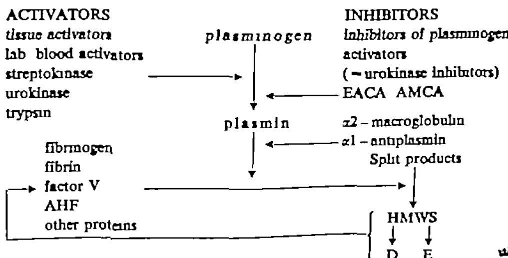
The women thus received a deep, intramuscular injection of 150 mg of Depo-Provera once every 12th week. At each visit they were examined gynecologically. The examination included also breast palpation, routine blood tests, weighing and history taking. All the women reported regular sexual activity throughout the observation period. All examinations were made by the same physician. For

Gynaecologists

Menorrhagia may be caused by an increase in local fibrinolytic activity Cyklokapron reduces menorrhagic haemorrhages by an average of 50 %

Women with average menstrual blood losses of over 80 ml have higher concentrations of plasminogen activators in the endometrium than those with lower blood losses. The resultant increase in local fibrinolytic activity is inhibited by Cyklokapron. The recommended dosage of Cyklokapron in menorrhagia is 1 g 3–6 times daily for 3–6 days With a dosage of 3 g daily Nilsson and Rybo noted reductions in bleeding *of 38 % compared with control cycles.* With twice this dosage bleeding was reduced by 51 %. None of the 36 patients participating in the trial were obliged to discontinue treatment as a result of side-effects.

Reference: NILSSON L., RYBO G. Treatment of menorrhagia with an antifibrinolytic agent, tranexamic acid (AMCA) A double blind investigation. Am. J Obstet. Gynecol 110 (1971) *p 113*



was never below normal levels. Altogether 17 women were given 75 µg of ethinyl-estradiol per day for 7–10 days in order to terminate prolonged bleeding or to induce withdrawal bleeding in cases of amenorrhea in women who feared that they had become pregnant. In all of the remaining 106 women, i.e. those who did not discontinue the trial because of the bleeding or amenorrhea and who did not receive estrogen therapy the irregularity of the bleeding was well tolerated without any special treatment.

Women using Depo-Provera generally bled quite often in the 12-week period after the first and second injections, but on most days bleeding was very scanty (spotting). The number of days of spotting decreased with increasing number of injections until most women were amenorrhoic. Table V gives the number of days of bleeding per 12-week period for those women who had kept menstrual calendars and who completed the trial without having received estrogens on any occasion.

It is evident that the number of days of bleeding declined with the increase in the number of injections.

Table V *The percentage of women still on trial with various numbers of days of bleeding in the approx. 12 week period after each injection*

After injections	Number of days of bleeding						No. of women
	>60	60–41	40–21	20–11	10–1	0	
1	—	11.3	20.0	31.3	25.0	12.5	80
2	—	4.1	20.3	14.9	31.1	29.7	74
3	1.6	3.2	9.5	14.3	28.6	42.9	63
4	—	—	6.0	8.0	30.0	56.0	50
5	—	2.6	7.7	7.7	5.1	77.0	39

Table VI *The percentage of women with various numbers of days of bleeding after each injection, among those who dropped out of the trial because of irregular bleeding*

After injections	Number of days of bleeding						No. of women
	60	60–41	40–21	20–11	10–1	0	
1	33.3	26.7	33.3	6.7	—	—	15
2	28.6	14.3	57.1	—	—	—	7
3	—	—	100.0	—	—	—	1
4	—	—	100.0	—	—	—	1

Table VII *a. Paired comparisons between initial weight and weight after 2 4 and 6 12-week periods*

	12-week periods		
	0–2	0–4	0–6
	97	55	13
Range (kilos)	−6.1–+8.7	−6.2–+12.2	−5.1 +5.4
t	3.12	4.31	1.74
p	<0.005>0.001	<0.001	>0.1
Significance			—

Table VII *b. Initial mean weight among the women and mean weight after 2, 4 and 6 12-week periods*

	12-week periods			
	0	2	4	6
	99	97	55	13
Mean (kilos)	60.9	61.5	63.0	59.3
S.D	11.3	11.4	13.3	9.7

For comparison, the bleeding pattern reported by patients who had dropped out because of bleeding is given in Table VI.

The women who discontinued the trial because of bleeding did not differ significantly in parity or age distribution from the group as a whole.

Weight gain was rather common in the series and was the second most common reason why the women discontinued the trial. Paired comparisons between initial weight and weight after 2, 4 and 6 12-week periods and the mean weight on those occasions are given in Tables VII *a* and VII *b*.

As for other side-effects given as reasons for discontinuation of the injections, it was often difficult to decide whether depression, minor psychological symptoms or depressed libido should be ascribed to the injections or to other factors. It might be mentioned that 17 of the 139 women reported improved libido during treatment, and they probably did so because they felt that they no longer feared becoming pregnant.

All endometrial biopsy specimens obtained showed pronounced atrophic mucosa with straight, sparse endometrial glands. In some cases these glands contained isolated drops of secretion, but most of them were inactive. The appearance of the biopsy specimens did not vary with the number of injections the women had received. (The

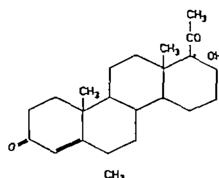


Fig 1 Medroxyprogesterone.

practical reasons it was not possible to give each injection after an interval of exactly 12 weeks. The injections were then given up to a week before the scheduled time.

Endometrial biopsy specimens were obtained from 15 women after a varying number of injections.

After a varying period, blood samples were obtained from 25 women for determination of plasma oestrogen levels.

Information on the subsequent menstrual cycle in women who discontinued the trial was obtained t revisits or by telephone. It was not always possible to obtain objective evidence of normal ovulation, i.e. basal temperature recordings or endometrial biopsy

Table I. *Age of the women*

Age in years	<20	20–24	25–29	30–34	35–39	>40
No. of women	8	25	57	22	21	6

Table II. *Fertility of the women*

No. of pregnancies	0	I	II	III	IV	>V
No. f women	13	19	36	30	17	24

Table III *Number of injections*

No. of injections	1	2	3	4	5	6	7	8
No. of women	20	32	19	19	18	19	10	2

Table IV *Cause of "drop-outs" and number of injections given before withdrawal*

Reason	Stopped after *n* injections 1	2	3	4	5	6	Total	%
Irregular bleeding	9	7	1	2	—	—	19	13.7
Weight gain	—	5	—	3	—	—	8	5.8
Loss of libido	2	1	1	—	—	—	4	2.9
Depression, minor psychological symptoms	—	1	1	2	—	—	4	2.9
Amenorrhoea	1	1	1	—	—	—	3	2.2
Migraine	—	—	1	—	—	—	1	0.7
Never returned for follow-up	3	1	—	—	—	—	4	2.9
Non-medical causes e.g. moved from the area, desires child, no partner	3	6	4	3	1	2	19	13.7
Total	18	22	9	10	1	2	62	44.6

RESULTS

None of the women became pregnant during the trial. Owing to unfortunate circumstances, one patient received her first injection of Depo-Provera when she was already in the 8–9th week of pregnancy which was not diagnosed until her visit for injection no. 2. The course of her pregnancy was uneventful and she was delivered of a healthy child. The patient was excluded from the series.

Of the original 139 participants, 6? (44.6%) had discontinued treatment by April 15 1971 (Table IV) 39 (28.1%) of the 62 had stopped because of symptoms which they ascribed to the injections. 19 (13.7%) of the 62 who had not experienced any side-effects stopped for other non medical reasons. 4 of the patients never returned for the scheduled check-ups and did not reply to written inquiries.

As expected from similar investigations on record, the reasons why the women discontinued the trial were mostly repeated spotting and/or unpredictable menstrual bleeding or amenorrhea (Table IV). In fact all the women reported some menstrual disturbance. After the first injection the cycles became quite irregular and the duration of bleeding totally unpredictable yet only about 15 % discontinued treatment because of irregular bleeding.

The bleeding was rarely profuse. The hemoglobin level, which was determined at each visit,

as never below normal levels. Altogether 17 women were given 75 μg of ethinyl-estradiol per day for 7–10 days in order to terminate prolonged bleeding or to induce withdrawal bleeding in cases of amenorrhea in women, who feared that they had become pregnant. In all of the remaining 106 women, i.e. those who did not discontinue the trial because of the bleeding or amenorrhea and who did not receive estrogen therapy the irregularity of the bleeding was well tolerated without any special treatment.

Women using Depo-Provera generally bled quite often in the 12-week period after the first and second injections, but on most days bleeding was very scanty (spotting). The number of days of spotting decreased with increasing number of injections until most women were amenorrhoic. Table V gives the number of days of bleeding per 12-week period for those women who had kept menstrual calendars and who completed the trial without having received estrogens on any occasion.

It is evident that the number of days of bleeding declined with the increase in the number of injections.

Table V. *The percentage of women still on trial with various numbers of days of bleeding in the approx. 12 week period after each injection*

After injections	Number of days of bleeding						No. of women
	>60	60–41	40–21	20–11	10–1	0	
1	—	11 3	20 0	31 3	25.0	12 5	80
2	—	4 1	20 3	14.9	31 1	29 7	74
3	1 6	3 2	9 5	14 3	28 6	42.9	63
4	—	—	6 0	8 0	30 0	56 0	50
5	—	2.6	7 7	7 7	5 1	77.0	39

Table VI. *The percentage of women with various numbers of days of bleeding after each injection, among those who dropped out of the trial because of irregular bleeding*

After injections	Number of days of bleeding						No. of women
	60	60–41	40–21	20–11	10–1	0	
1	33 3	26.7	33 3	6 7	—	—	15
2	28 6	14.3	57 1	—	—	—	7
3	—	—	100 0	—	—	—	1
4	—	—	100 0	—	—	—	1

Table VII *a*. *Paired comparisons between initial weight and weight after 2, 4 and 6 12-week periods*

	12-week periods		
	0–2	0–4	0–6
	97	55	13
Range (kilos)	−6 1–+8.7	−6 2–+12.2	−5.1–+5.4
t	3.12	4 31	1 74
p	<0.005>0.001	<0.001	>0.1
Significance			—

Table VII *b*. *Initial mean weight among the women and mean weight after 2, 4 and 6 12-week periods*

	12-week periods			
	0	2	4	6
	99	97	55	13
Mean (kilos)	60.9	61.5	63 0	59.3
S.D.	11.3	11 4	13.3	9 7

For comparison the bleeding pattern reported by patients who had dropped out because of bleeding is given in Table VI.

The women who discontinued the trial because of bleeding did not differ significantly in parity or age distribution from the group as a whole.

Weight gain was rather common in the series and was the second most common reason why the women discontinued the trial. Paired comparisons between initial weight and weight after 2, 4 and 6 12-week periods and the mean weight on those occasions are given in Tables VII *a* and VII *b*.

As for other side-effects given as reasons for discontinuation of the injections, it was often difficult to decide whether depression, minor psychological symptoms or depressed libido should be ascribed to the injections or to other factors. It might be mentioned that 17 of the 139 women reported improved libido during treatment, and they probably did so because they felt that they no longer feared becoming pregnant.

All endometrial biopsy specimens obtained showed pronounced atrophic mucosa with straight, sparse endometrial glands. In some cases these glands contained isolated drops of secretion, but most of them were inactive. The appearance of the biopsy specimens did not vary with the number of injections the women had received. (The

Table VIII *Subsequent bleeding pattern of 31 of the women who stopped Depo-Provera treatment*

	Number of months after the last 12 week period on Depo-Provera						Total no. of women	Preg-nancies
	1	2	3	4	5	6		
Regular cycles + secretory endometrium (PAD)	—	—	—	1	—	—	1	—
Regular cycles + biphasic basal body temp.	2	—	—	1	—	—	3	1
Regular cycles	14	5	4	3	—	—	26	3
Irregular bleeding	—	—	—	—	—	1	1	—

specimens were scrutinized by Dr Inga Hägerstrand, Institute of Pathology Malmö General Hospital.)

Only relatively few determinations were made of the plasma estrogen (made by Prof Elof D B Johansson Uppsala) However practically all of those determinations made 7–10 days after an injection of Depo-Provera were clearly below normal values for women in early follicular phase. Determinations made in the middle or at the end of a 12 week period bordered the lower limit of the normal range or were clearly within normal limits. The number of determinations was, however too small to warrant statistical analysis.

Routine palpation of the breast and ordinary gynecological examination of all the women at each follow-up revealed nothing remarkable

Of the 62 women who stopped using Depo-Provera 47 could be successfully contacted after varying periods of time since the last scheduled visit. Sixteen of them reported that they had immediately started using a combined type of oral contraceptive without waiting for spontaneous menstruation Information on the subsequent menstrual cycles in the other 31 women is given in Table VIII

Three patients discontinued the trial because they wished to have children. Two of these women have in the meantime become pregnant but not the third who however has menstruated regularly for the last 12 months. One of the two women who became pregnant aborted in the 2nd or 3rd month the other is expected to be delivered at the time of writing. Two more pregnancies occurred in the group 1 month and 12 months, respectively after the effect of the last injection had worn off Both pregnancies were unplanned and were interrupted by legal abortion.

Those patients who received their first injections post-partum did not feel that the secretion of milk was less than during previous periods of lactation.

DISCUSSION

Depo-Provera in a dose of 150 mg i.m every 12th week is generally believed to exert its contraceptive effect by inhibiting ovulation (11 20). The gonadotrophin pattern during the use of Depo-Provera suggests a constant inhibition of the preovulatory LH peak, while the basal LH secretion and FSH production appear not to be affected (13) Even after long treatment with Depo-Provera the microscopic examination of the ovaries shows growth of primordial follicles up to the stage of graafian follicles (20) and the theca interna shows evidence of luteinization. Both findings suggest FSH and LH activity though not sufficient to induce ovulation.

Like other gestagenic contraceptives Depo-Provera has also an effect on the cervical mucosa and endometrium (12, 20) which increases its contraceptive effect.

As expected from published investigations (Table IX) the frequency of unplanned pregnancy in this material was low

Upjohn's compilation of series studied in June 1965 to June 1969 mainly in the U.S. comprises 3 188 women observed for more than 50 000 woman-months. During this period 10 women became pregnant, i.e. a Pearl index of 0 24

Table IX. *Reports of effectiveness in some earlier investigations*

Authors	Number of pregnancies	No. of woman-months observed	No of women
Powell & Seymour (USA)	1	14 000	1 123
Soichet (USA)	1	4 200	300
Mishell (USA)	0	3 577	340
Tyler et al. (USA)	0	2 641	226
Marker (Denmark)	0	1 430	237

Table X. *Advantages of pure gestagen contraception among 77 women who without success had tried oral contraceptives containing oestrogen before Depo-Provera*

Reasons for stopping oral contraception with combined type of pills		During Depo-Provera treatment		
		Better than on pills	Same side-effect as on pills	Stopped Depo-Provera because of other side-effect than on pills
Discomfort, turgidness	18	12	—	2 loss of libido 3 weight gain 1 irregular bleeding *6*
Nausea	16	12	—	4 irregular bleeding *4*
Weight gain	16	8	4	1 irregular bleeding 1 depression 1 migraine 1 loss of libido *4*
Depression, minor psychological symptoms	12	9	2	1 irregular bleeding *1*
Impaired feeling of well-being	6	4	—	2 irregular bleeding *2*
Headaches	4	4	—	
Loss of libido	3	—	2	1 depression *1*
Retention of body fluid	2	2	—	
Total	*77*	*51*	*8*	*18*

Though the ordinary regular menstrual pattern invariably disappeared, only about 15% of the women discontinued treatment because of unpredictable bleeding or amenorrhea. This drop-out because of bleeding disturbances is roughly the same as that found in trials with oral low doses of gestagens (6, 7), in which intermenstrual bleeding was also initially common. Before treatment with Depo-Provera the patient should be thoroughly informed about the expected effect on the menstrual cycle. There is not even an initial intent to maintain what might be *normal cycles and it must be accepted, to begin with, that* there are going to be menstrual disturbances (19). Reduction of the number of days of bleeding in patients on Depo-Provera by treatment with a daily dose of oral estrogen has proved discouraging (2). It is true that when the estrogen was given not continuously but for 21-day periods followed by 7-day pause, withdrawal bleeding occurred regularly. But since this involved loss of most of the advantages of the method, the authors felt that but little could be gained by the use of an oestrogen supplement by patients receiving Depo-Provera (2).

The question has often been raised whether these women, anovular during treatment and not given exogenous oestrogen supplement, might suffer from subclinical hypoestrogenism which may with time become harmful (12). The appearance of endometrial biopsy specimens as well in our investigation as in earlier series (13) suggested a reduced oestrogenic effect on the endometrium, an assumption corroborated by examination of vaginal smears. It has also been discussed whether this progestational dominance in effector organs might be due to potent, local anti-estrogenic properties of medroxyprogesterone acetate or to a *pronounced general reduction of biologically active* estrogen in the blood (13). In the above-mentioned studies in which patients receiving Depo-Provera received also oral estrogens, the latter had no significant effect on the appearance of endometrial biopsy specimens or on vaginal smears (2). It is hoped that a more detailed investigation of the plasma estrogen will help to elucidate this point. The women had no menopausal symptoms, no atrophy of the breasts, no senile vaginitis and no predisposition to other types of vaginitis.

Slight weight gain was more or less the rule and weight gain was also the second most common reason why women discontinued treatment. Such

weight gain has also been found by among others, Spellacy et al. (18) who also claimed to have found a correlation between weight gain and a rise in the blood sugar level They also found a significant increase in the plasma insulin These effects on carbohydrate metabolism were ascribed to a glucocorticoid-like effect of the steroid. But in their study Depo-Provera was used in a dose of 400 mg every 6th month They consider these findings to indicate a careful study of the carbohydrate metabolism before recommending this form of contraception However such treatment had no effect on the plasma level of the growth hormone in that study

As expected from foreign reports, follow-up of the women who discontinued treatment with Depo-Provera confirmed that the amenorrhea induced by the contraceptive is reversible (Table VIII) Gardner & Mishell (3) reported signs of regular ovulation within 18 months of the last injection of Depo-Provera in 40 of 43 women. In a similar series of 27 women Tyler et al (19) found that all the patients ovulated within 12 months of their final injection but stated that subsequent ovulation was not always regular In neither of these series did the tim of recovery of ovulation depend on the number of injections given

The unpredictability of the return of a normal regular menstrual cycle, which may not occur until one year or more after the final injection makes this preparation most suitable for women who have decided not to have any more children. Other women who may later wish to give birth should stop using Depo-Provera at least 6 months to 1 year before planning a new pregnancy Most patients who have tried to conceive immediately after termination of Depo-Provera therapy did usually succeed within 1 year after their final injection (13 15) but such series are still rather small.

Pure gestagens are considered superior to preparations containing oestrogens with regard to their effects on plasma protein levels. Pronounced disturbances of the normal plasma protein pattern are seen in women on combined estrogen gestagen preparations (8) while pure gestagen in low doses does not cause such disturbances (9).

The undesired side-effects of conventional oral contraceptives on women with a history of liver disease are likewise ascribed mainly to estrogens, or estrogens in combination with gestagens. In a recent investigation (16) of a number of patients with hepatosis of pregnancy (pruritus with raised GPT and/or GOT) treatment with various gestagenic or estrogenic steroids or combinations of both were carried out. Preparations containing estrogens were found to cause abnormal liver function tests, while pure gestagens had no such effect

Hormonal contraceptives also have been reported to predispose to thrombosis mainly because of the estrogenic component (4). Thus, Daniel (1) showed that the use of estrogenic hormones to stop lactation increased the risk of thrombosis. In a study of the fibrinolytic system in women given ethinyl estradiol prior to surgery for prolapse Åstedt (21) found the activators of fibrinolysis in the vein walls to be depressed a change which has been shown to predispose to thrombosis (5)

On the other hand examination of the coagulation factors and the fibrinolytic system during continuous daily administration of chlormadinon-acetate in a dose of 0.5 mg disclosed no changes (14) Similar studies of more than 20 women treated with Depo-Provera have also failed to reveal any changes that might predispose to thrombosis (Jeppsson S. & Åstedt, B Unpublished data)

A search of the literature failed to reveal any evidence suggesting that treatment with pure gestagenic hormones predisposes to thrombosis. Further development of contraceptives containing pure gestagens would therefore appear desirable.

Many of the women who took part in the Depo-Provera trial had previously used combined types of oral contraceptives and often found them unsatisfactory Since they wished to continue hormonal contraception, because of its effectiveness, they were invited to participate in the Depo-Provera trial. As seen in Table X 51 (66.2%) of the 77 women who had previously never been able to tolerate oral contraceptives were very satisfied with Depo-Provera The remaining 26 patients stopped using Depo-Provera either because of the same side-effects as those of oral contraceptives, i.e. mainly weight gain emotional distress and depression of libido or other undesired reactions.

The investigation thus confirmed that the preparation is reliable and with the exception of the unpredictable menstrual pattern the method ap-

pears to produce only few side-effects. An obvious advantage of the method is its safety in particular it avoids the risk of accidental failure among forgetful women using oral contraceptives. Many of the women in the study had a history of multiple legal abortions and many multiparous women who had previously failed in the use of various kinds of contraceptives. Many of these women found Depo-Provera satisfactory and the method appears well-suited to this type of patient. Moreover pure parenteral or oral gestagenic contraceptives not combined with estrogens, appear not to be predisposing to thrombosis or to impair liver function. All this, together with the fact that even in this study there were a relatively large number of women who could not tolerate conventional, oral contraceptives of combined type but who were very satisfied with Depo-Provera, suggests that the method should be incorporated in our current contraceptive armamentarium, especially as an alternative for mainly somewhat older women who desire no more children and who have found other methods of contraception unsatisfactory

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PERFUSION OF THE ISOLATED HUMAN UTERUS

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Abstract. The authors developed new machine for organ perfusion and by using this succeeded in preserving an isolated human uterus in condition of normal body temperature and humidity of 100 percent.

Moreover, the authors succeeded in preserving a trophoblastic tumor in utero.

The following conclusion can be made from such experiments.

1. Concerning the machine, (*a*) The use of fluid amplifier gives long durability and allows long operation of the machine. (*b*) Blood destruction is negligible with the diaphragm-pump. (*c*) The flow rate and pressure of the perfusate can be controlled easily by changing the supply-pressure and the stroke of pump. (*d*) The wave form of the pulse can be made to approximate that in the living body. (*e*) The temperature of the perfusate is controlled automatically to normal body temperature. (*f*) The input and output of the perfusate are balanced by control mechanism.

2. Concerning the experimental results (*a*), the authors succeeded in preserving an isolated human uterus for more than 3 hours, at normal body temperature. (*b*) The most useful tests of uterine function are the electromyogram, the lactate- pyruvate concentration and ratio. (*c*) The authors also succeeded in preserving trophoblastic tumor in the isolated uterus. In this experiment, was also useful to test the HCG and HPL concentration in the perfusate and to study tritiated thymidine radio-autograms of the tumor cells. (*d*) Prostaglandin $F_{2\alpha}$ is very useful for the study of the activity of the isolated human uterus.

The methods most frequently used to investigate the mechanisms of human reproduction include the analyses of hormone levels in blood or urine and histochemical examination of endometrium and various endocrine organs.

On the other hand, techniques of molecular and cellular biology also are employed in studying reproduction.

By these indirect methods, however it is impossible to elucidate the function of the reproductive organs, especially of the human uterus which is the main organ of reproduction.

In order to approach the problems of human reproduction more directly the authors developed a new machine for organ perfusion and tried to use it for perfusion of the isolated human uterus.

In this paper the authors describe a new experiment and some interesting results from the study of organ biology"

I. *Short Description of the Machine for Organ Perfusion*

As the details of the mechanism and function of this newly developed machine has been reported already in the preliminary paper (3 5) we intend to outline only the main features of the machine.

This machine was so constructed that it might be applied to various experiments and it consists of an artificial heart pump with diaphragm moved to and fro by a liquid amplifier and includes also a compliance mechanism for regulating pressure wave forms, a synchronizer an organ chamber a suction pump, an oxygenator with three discs, heating chamber connected to a thermostat, completing the automatic control system for regulating the temperature and pressure of the perfusate to that found in the human uterus in vivo.

As can be seen in Fig. 1 our machine has a special type of artificial heart which is composed of a diaphragm-pump connected to a liquid amplifier moved by high air pressure from an air compressor

The merit of this fluid amplifier is that it has a long durability because it has no moving part, and that its function is not changed by drastic changes of temperature by chemical substances or by radioactivity

Moreover the destruction of blood cells is minimal because of the mechanism of the pump.

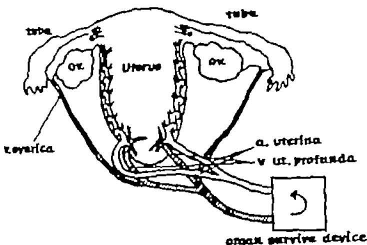


Fig 3 Diagram of extracorporeal perfusion of the human uterus and its vascular system.

that of uterine arteries in vivo in order to preserve organ function for a long time

Therefore in our machine we included a very efficient compliance mechanism for wave form regulation.

II. *The Perfusate*

Usually we use Hanks solution containing 20% blood and 4% dextran.

Since about 300 ml of the perfusate is sufficient to operate the machine, we use about 60 ml of patient's blood, and sometimes urokinase or heparin to prevent blood coagulation in the machine, and during experiments, besides the determination of erythrocyte count, hematocrit (Ht), hemoglobin content (Hb) PO_2 and PCO_2, usually use the serial estimations of GOT and GPT activities (S. Reitman et al. Amer J Clin Path 28. 56 1957) lactate concentration (H U Bergmeyer Method of Enzymatic Analysis, 266, 1965 Acad Press, N Y) pyruvate concentration (S Shibata The Technique of Clinical Examination 214 1960 Kanehara, Tokyo) of the perfusate

Moreover in the experiments on the uterus containing a trophoblastic tumor radioimmunoassay of HCG and HPL of the perfusate is used to determine the endocrine activity of the trophoblastic tissue.

At this time we use A. R. Midgley's method (J Clin Endocrinol 26. 1375 1966) for HCG-assay with antigen which was highly purified and standardized with the Second International Standard HCG in our laboratory (Y Ashitaka et al. Endocrinology 87 2, 233 1970), and modified F C. Greenwood and W M. Hunter's method (Biochem J 89 114 1963) for HPL-assay with highly purified antigen prepared also in our laboratory (H. Morikawa et al. Endocrinol Jap 118 5, 1971)

The data obtained are shown below under heading IV

III. *Use of Hysterectomy Specimen in Machine*

For the experiment, we usually use the human uterus along with both tubes and ovaries removed by a simple abdominal operation. During the operation the uterine arteries and veins in

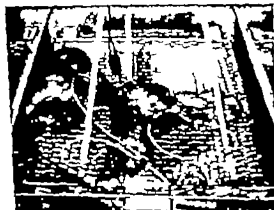


Fig 4 Human uterus with trophoblastic tumor in the organ chamber (electrode is inserted at the site of uterotubal junction).

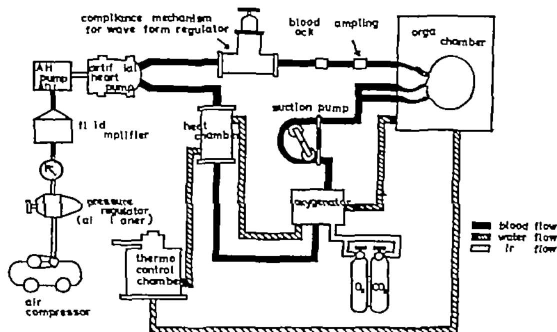


Fig 1 The schematic presentation of the machine

The perfusate which is pushed out from this pump flows into the compliance mechanism for wave form regulation and the pressure wave form is made to simulate that in the uterine arteries in vivo (Fig. 2)

The pulse rate is also controlled from 50 to 120 per minute depending on the purpose of the experiments.

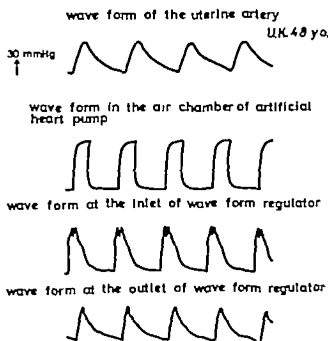


Fig 2 Comparison of wave forms between uterine artery in vivo and various points of the machine.

The perfusate from the wave form regulator flows into the arteries of the surgically removed uterus set in the organ chamber which is regulated at the normal body temperature (36.5–37.0 C) and a humidity of 100%

After perfusing the whole organ the perfusate is gently sucked up into the oxygenator by a roller type of suction pump from the uterine veins and the ovarian veins in infundibulopelvic ligaments.

The perfusate is oxygenated completely in the *oxygenator with three discs, and flows on into* the heat chamber (36.5–37.0 C) which is controlled by a thermostat as we can see in Fig. 1

Next the perfusate warmed up to normal body temperature and well oxygenated, is led once again into the artificial heart system.

It can be seen that the device has four special systems.

1 Artificial nervous system. electric autoregulatory mechanism,
2. Artificial blood vessel system: reasonably normal pathway of the perfusate through the uterus,
3 Heating system. thermostat for heating the perfusate,
4 Air system. efficient oxygenation mechanism and air compression mechanism to promote the regular movement of the artificial heart.

Moreover it is very important to make the pressure wave form of the perfusate similar to

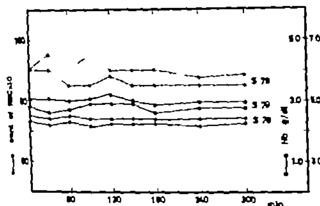


Fig. 6. Erythrocyte count, Hb and Ht in the perfusate.

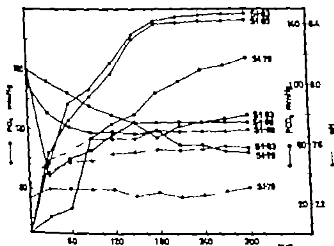


Fig. 7. PO_2, PCO_2 and pH of the perfusate.

tient oxygenation during perfusion for at least 5 hours is recognized.

The PCO_2 is rather high and pH is low just after the beginning of perfusion but before long

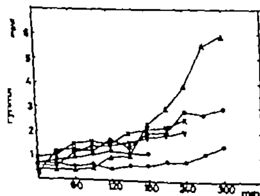


Fig. 8. Pyruvate in the perfusate. Δ—Δ failed case in preservation.

both reach the normal level and remain reasonably constant during perfusion.

3. *Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT)*

GOT and GPT values do not change much during perfusion. From our results, we conclude that GOT and GPT are not such good tests of the functional activity of the perfused organ.

4. *Pyruvate, lactate concentration and ratio*

Figs. 8 and 9 show changes in the pyruvate and lactate concentrations. Usually only very gradual increases in the levels of these substances are recognized and where they rise rapidly the extracorporeal preservation of the organ fails as a result.

The same thing is true for change in the lactate pyruvate ratio as is shown in Fig. 10.

Table I. *Normal values necessary for Uterine Perfusion*

Pressure of the uterine artery			
contracted		120–150 mmHg	
flaccid		60–80 mmHg	
Flow rate in uterine artery unilaterally		25–40 ml/min	
Temperature		37°C	
pH and partition pressures of gases in the uterine vessels			
	pH	PO_2	PCO_2
uterine artery	7.38	140 mmHg	52 mmHg
uterine vein	7.3?	78 mmHg	56 mmHg

the broad ligament are preserved as long as possible to be connected with the artificial blood vessel system of the machine

The venous system of the uterus, especially the plexus round the cervix and upper vagina is so complicated that finding and preserving the main veins along the lateral wall of the cervix is not easy

At the same time as main venous outflow of the uterine corpus is through the pampiniform plexus in the infundibulopelvic ligament we dissect the ligament nearly to the pelvic wall.

Thus, we use two arteries (bilateral uterine arteries) and four veins (bilateral uterine veins and ovarian veins) for connecting the uterus to the artificial blood vessel system in the machine.

Just after removal, the uterus is immediately perfused through the arterial stumps with a cold balanced electrolyte solution containing 2.5% of low-molecular dextran, 150 mg% of glucose, 2 mEq/l of magnesium sulfate and 6 mg/kg of heparin, buffered to a pH of 7.45 and then the uterine arteries are connected to the artificial arteries and the uterine veins and ovarian veins are also connected to the artificial vein system in the machine as we can see in Fig. 3

Fig. 4 shows a picture of the uterus containing a trophoblastic tumor including the bilateral ovarian lutein cysts in the organ chamber and connected completely with the vascular system of the machine.

The conditions which are usually necessary for maintaining the uterus are shown in Table I.

IV. *Tests of Uterine Function*

In order to learn as much as possible about the isolated organ (1 3 4) we apply routinely many physiological, biochemical and histochemical tests of uterine function and use them to assess how function has been preserved.

1 *Electromyogram during perfusion*

As is shown in Fig. 4 we routinely place an electrode at the site of the uterotubal junction and try to detect the electrical depolarization in the myometrium during perfusion.

Fig. 5 shows an electromyogram from an isolated uterus. The typical initial depolarization is seen, and after injection of phostaglandin $F_{2\alpha}$ into the perfusate regular spikes are recognized very clearly

On the other hand, rather large amounts of oxytocin induce very strong and irregular depolarization even when 5 hours have passed since the beginning of extracorporeal perfusion.

Thus the electromyogram is one of the most important tests of uterine function in this experiment.

2 *Erythrocyte count hematocrit (Ht) hemoglobin content (Hb) PO_2, PCO_2 and pH*

As we can see in Fig. 6, erythrocyte count, Ht and Hb do not change, and in Fig. 7 very effi-

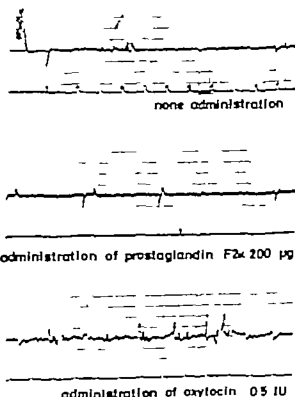


Fig. 5 Electromyogram of the human uterus during perfusion.

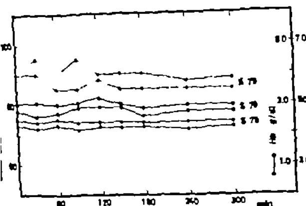


Fig. 6. Erythrocyte count, Hb and Ht in the perfusate.

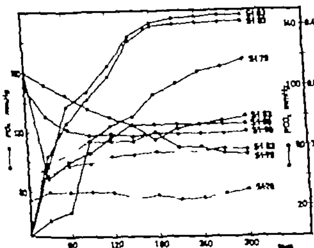


Fig. 7. PO_2, PCO_2 and pH of the perfusate.

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The PCO is rather high and pH is low just after the beginning of perfusion but before long both reach the normal level and remain reasonably constant during perfusion.

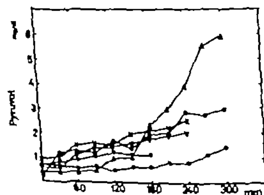


Fig. 8. Pyruvate in the perfusate. Δ—Δ, failed case in preservation.

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GOT and GPT values do not change much during perfusion. From our results, we conclude that GOT and GPT are not such good tests of the functional activity of the perfused organ.

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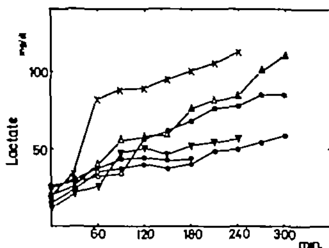


Fig 9 Lactate in the perfusate. x — x failed case in preservation.

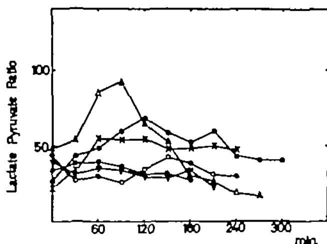


Fig 10 Lactate pyruvate ratio in the perfusate. Δ—Δ failed case in preservation.

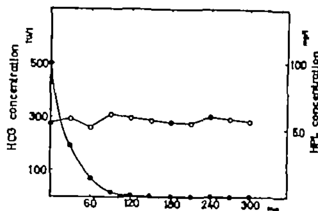


Fig 12. HCG and HPL in the perfusate, without organ. Perfusate contains exogenous HCG and HPL.

From the above results, we can say that the most useful chemical tests of function in the perfused organ are pyruvate and lactate concentration and their ratio.

5 *Microangiogram of the perfused organ*

Fig. 11 is a microangiogram of the isolated uterus just after the end of experiment. We cannot find any damage in the vascular system of the uterine wall

V *Experimental Perfusion of Uterus Containing a Trophoblastic Tumor*

In this experiment, as well as the tests listed above we measured changes in human chorionic gonadotrophin (HCG) and human placental lactogen (HPL) levels in the perfusate by highly sensitive radioimmunoassay



Fig 11 Microangiogram of the preserved human uterus for 5 hours.

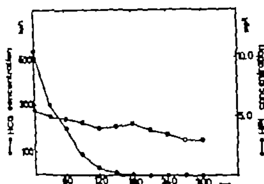


Fig. 11. HCG and HPL in the perfusate, with myomatous uterus. Perfusate contains exogenous HCG and HPL.

Moreover the DNA synthesizing activity of the tumor cells was also checked by tritiated thymidine radioautography of tumor tissue which was taken by repeated biopsy during the perfusion.

1. *Changes in HCG and HPL levels during perfusion*

Before the main experiment, we did some preliminary studies of the denaturation of these hormones.

It is well known that HCG is glycoprotein which is resistant to the extrinsic influences. But HPL, which is a simple protein, is very labile and is denaturated or destroyed very easily by many factors.

Fig. 12 shows the results of an experiment in which the machine was operated without connection to the uterus.

The level of HCG in the perfusate does not change much, but added HPL disappears very rapidly from the perfusate.

Fig. 13 shows the results of an experiment with an isolated myomatous uterus.

In this case at the beginning of the perfusion, HCG and HPL were added to the perfusate.

HPL disappears rapidly but the HCG level falls very gradually. HPL is destroyed very rapidly in the myomatous uterus, but HCG is not so easily denatured.

After these preliminary experiments, we tried to perfuse uterus containing a trophoblastic tumor and to study the endocrine function of the tumor in the extracorporeal state.

Fig. 14 shows the results of this experiment.

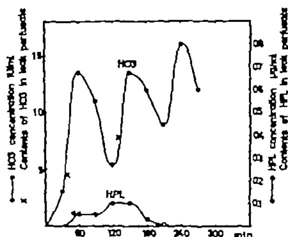


Fig. 14. HCG and HPL in the perfusate. Uterus with trophoblastic tumor.

It is obvious that the level of HCG in the perfusate increases rapidly making periodic peaks.

On the contrary HPL makes only one low peak and disappears from the perfusate after 3 hours.

From this characteristic pattern of HCG in the perfusate, we may conclude that during perfusion the trophoblastic tumor cells continue to function and actively release HCG into the perfusate.

2. *Effect of actinomycin D on a trophoblastic tumor during perfusion*

In another experiment with a trophoblastic tumor we tried to study the effect of actinomycin D on the isolated tumor.

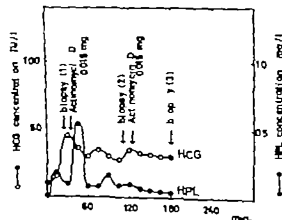


Fig. 15. Influence of actinomycin D on the levels of HCG and HPL in the perfusate. Uterus with trophoblastic tumor.



Fig 16. ^{3}H-thymidine uptake f preserved tumor cells (before actinomycin injection).

In this experiment, we made repeated biopsy of the tumor tissue during perfusion and examined the changes of DNA synthesizing activity of the tumor cells by radioautography

As Fig. 15 shows, just after the injection of actinomycin D into the perfusate, the peaks of HCG production cease.

These findings are completely different from those in the initial experiment without actinomycin D and suggest that actinomycin D attacked the trophoblastic tumor and supressed hormone production.

Fig. 16 shows a ^{3}H-thymidine radioautogram of tumor tissue just before an actinomycin injection. Very active DNA synthesis is seen in tumor cells.

Fig. 17 is a radioautogram which is taken after the second injection of actinomycin D Thymidine uptake is very slight.

From these experiments, we conclude that with our machine we can preserve not only the human uterus itself but also a trophoblastic tumor for 5 hours or more in the extracorporeal state, and we may be able to study uterine function, the endocrine function of the trophoblast, and moreover the direct actions of anti-cancer agents on uterine tumors at the organ level

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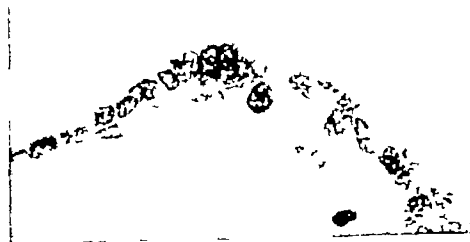


Fig 17 ^{3}H-thymidine uptake of preserved tumor cells (after actinomycin injection).

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MALIGNANT TROPHOBLASTIC NEOPLASIA

Monitoring of Therapy

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Abstract. Malignant trophoblastic neoplasia is uncommon in Scandinavia, only 4–5 cases per 100 000 live births being reported to the National Cancer Registries each year. In a small series comprising 30 patients the effectiveness of chemotherapy in curing non-metastatic as well as metastatic disease was confirmed. Of 16 women with metastatic choriocarcinoma, 12 were cured by combined treatment with methotrexate and actinomycin D. In the monitoring of treatment and follow-up of the patients radioimmunoassay of LH in serum was found more sensitive and reliable than determination of HCG in 24-hour urine. A delayed response to chemotherapy with fluctuating hormone levels during chemotherapy was observed to be of prognostic and therapeutic significance.

In Scandinavia as in other Western countries the incidence of malignant trophoblastic neoplasia (MTN) is very low. Only 3–4 cases are reported to the Cancer Registry of Norway each year (3) which gives an incidence of approximately 5/100 000 live births. The corresponding figure for Sweden is 4/100 000 (11). It is evident that without centralization of treatment, it is impossible to obtain any real personal experience of this rare tumour type. In Norway during the last few years almost all patients with a suspicion of MTN have been referred to our institution, and in the period 1964 through 1970 we have treated altogether 30 cases of MTN. Although the observation period is too short for an exact evaluation of the final result, some of our experiences, particularly with chemotherapy, may be of interest.

MATERIAL

According to the definitions recommended by the International Union against Cancer (4), all cases of MTN should be classified as non-gestational or gestational, non-metastatic or metastatic. The present series of 30 cases all developed after pregnancy and in 16 cases metastases were found.

The histological diagnoses were made on the following criteria (10):

Choriocarcinoma (CC) was diagnosed in hysterectomy specimens and in metastatic deposits on the commonly accepted microscopic findings of trophoblastic malignancy in the absence of villi. In curettings, where invasion may not be evident, the diagnosis was made if the tissue consisted entirely of non-villous malignant trophoblast with or without uterine muscle.

Invasive mole (IM) was diagnosed in hysterectomy specimens, metastatic deposits or in curettings showing infiltration by malignant trophoblastic tissue containing chorionic villi.

The advent of successful chemotherapy for MTN has resulted in some cases with definite clinical evidence of such a neoplastic process, but without histological confirmation of diagnosis. Such cases will in the following be classified as histologically uncertain.

The seven cases listed as histologically uncertain in the present series all developed after molar pregnancy (Table I). The diagnosis of a possible malignant trophoblastic tumour was in all cases made on the basis of the past history, persistently elevated gonadotrophin titre, and pathological arteriogram.

Of the total series of 30 cases, 19 developed after hydatidiform mole, 8 after abortion and 3 after term pregnancy. It appears from Table I that MTN developing after abortion or term pregnancy seems to run a more malignant course than those cases developing after hydatidiform mole. It must be remembered, however, that the time interval to the diagnosis of MTN usually is much shorter for cases following molar pregnancy than for those developing after abortion or term delivery. The reason for this is that after the expulsion of molar tissue, the patient as a rule is followed thoroughly with pregnancy tests or determinations of chorionic gonadotrophin (HCG) in urine. Of the 4 cases in *Table I* with metastatic CC following molar pregnancy, 3 had an inadequate follow-up, and the ultimate diagnosis of MTN was made

Table I *Preceding pregnancy and histological diagnosis*

Histology	Preceding pregnancy *Mole*	*Abortion*	Term delivery	*Total*
Uncertain	7	—	—	7
Invasive mole	4	—	—	4
Choriocarcinoma	*1*	2	—	3
Metastatic choriocarcinoma	7	6	3	16
Total	19	8	3	30

Four cases without histological conformation of diagnosis.

more than 1 year after evacuation of a supposedly benign mole. Table II shows the interval between the preceding pregnancy and diagnosis of MTN in relation to the histopathological diagnosis and the occurrence of metastases. It is evident that making an early diagnosis is as important in MTN as in other fields of oncology The table also illustrates the well-known fact that in some cases metastases may develop extremely rapidly in the course of the disease.

The age distribution appears in Fig. 1 The youngest patient was 17 and the oldest 58 years of age at the time f diagnosis. Four patients were postmenopausal with an interval between the last menstrual bleeding and the *detection of MTN of from 1 to 4 years.*

METHODS

Monitoring of treatment was, in the earlier years of the study period, performed by determinations of HCG in urine with the haemagglutination inhibition method (13). Since 1968 radioimmunoassay of luteinizing hormone (LH) in serum (8 9) has also been used.

In cases treated by chemotherapy alone the effect of treatment upon the local disease in the uterus was followed by repeated pelvic rteriograms. The usefulness of this procedure has been discussed earlier (5).

General principles of treatment which have developed in the present series have been as follows:

Table II *Time interval from preceding pregnancy to diagnosis*

Histology	Time interval in months 2	3–5	6–11	1 23	24–48	Total
Uncertain	5	2	—	—	—	7
Invasive mole	3	1	—	—	—	4
Choriocarcinoma	—	*1*	—	*1*	*1*	*3*
Metastatic choriocarcinoma	3	2	2	4	5	16
Total	11	6	2	5	6	30

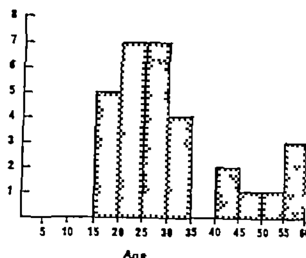


Fig 1 Age distribution. Three of the patients were postmenopausal.

Invasive mole localized to the uterus was usually treated with methotrexate (MTX) only Initially 25 mg was given in daily intramuscular injections for 3–5 days depending upon body weight, general condition and blood examinations. In an attempt to counteract the most unpleasant side effect which, in our experience has been stomatitis, the patient was encouraged to rinse her mouth at frequent *intervals for at least 1 hour immediately after* each injection, with a mouthwash consisting of 3 mg citrovorum factor (Leucovorin) and 1 000 I.U. hyaluronidase (Penetrase) dissolved in a glass of water

If there was manifest metastasis from an invasive mole or a histologically proven choriocarcinoma, in addition t methotrexate, 0.5 mg actinomycin D (Act. D) as given daily for 3–5 days. The drug was given intravenously in 1 000 ml saline over a period f at least 3 hours. The interval between each course f MTX or combined MTX/Act. D varied from 6 to 14 days depending upon toxicity symptoms and blood examinations. It was deemed desirable that the courses should follow one another as closely as possible, *especially in cases with widespread* metastases. The number of courses depended upon the effect of the treatment on the HCG or LH level in urine or serum. Gonadotrophin determinations were carried out each week during the treatment. A full blood count as done twice weekly differential count, and liver and kidney function tests once week.

In patients with anemia blood transfusions were always given before treatment started. As an adjuvant to chemotherapy and with the aim f *stimulating the bone marrow* and improving the general condition of the patients, 100 mg of an anabolic steroid, methenolone oenanthate (Primobolan® Depot, Schering), was administered each week, and the patients were kept on a diet rich in protein, minerals and itamins.

With increasing experience with chemotherapy surgical treatment involving hysterectomy was considered advisable only for complications such as excessi bleeding and per

foration of the tumour into the peritoneal cavity or if the primary tumour in the uterus showed poor response to chemotherapy as judged by serial pelvic arteriograms and hormone determinations.

RESULTS

Of the total series of 30 patients, 13 were treated with chemotherapy alone, one with hysterectomy and 15 with combined chemotherapy and hysterectomy. One patient with widespread metastases was admitted in an extremely poor general condition and died before the first course of chemotherapy could be completed.

It appears from Table III that MTX alone gave complete remission in altogether 8 cases, 7 of which were listed as histologically uncertain and 1 as metastatic choriocarcinoma. Combined MTX/Act. D-therapy was successful in 4 out of 6 cases with metastases. Review of the histological material showed that at least five of the hysterectomies performed in patients receiving pre-operative chemotherapy probably were unnecessary because only remnants of seemingly non-viable tumour cells were detected in the operation specimens. In 2 patients hysterectomy was carried out before admission to this unit. With our present knowledge it may be justifiable to suggest that probably 20 out of the 30 patients might have been treated successfully with chemotherapy alone.

On the other hand we have also experienced that surgery still has a definite place in the treatment of MTN. In 3 patients laparotomy had to be carried out because of tumour rupture with life-threatening bleeding into the peritoneal cavity. Moreover in 3 other patients hysterectomy was performed when resistance to chemotherapy seemed to develop.

The four treatment failures are of special concern and will be described in some detail:

Case 19 age 24, had one earlier pregnancy which ended in abortion 16 months before diagnosis of CC was made by curettage. Four courses of MTX were given at intervals of 7–10 days without any appreciable effect on the size of the tumour and hysterectomy was performed. No signs of metastases was found and the patient was discharged. Three months later she returned with multiple lung metastases which did not respond to MTX. At autopsy generalized disease was found, with dissemination to the liver, kidney, intestines, lungs, brain, and skeletal system.

Case 33 age 55 had two earlier pregnancies, the last of which was a hydatidiform mole at an age of 52 years.

Table III. *Survival in relation to histology and therapy*

Histology	Therapy	Total No. alive
Uncertain	MTX	7/7
Invasive mole	Surgery	1/1
	MTX + Surg.	2/2
	MTX + Act. D + Surg.	1/1
Choriocarcinoma	MTX + Surg.	1/1
	MTX + Act. D + Surg.	2/2
Metastatic choriocarcinoma	MTX	1/1
	MTX + Act. D	6/4
	MTX + Surg.	3/2
	MTX + Act. D + Surg.	6/5
Total		30/26

MTX: Methotrexate Act. D: Actinomycin D Surg.: Surgery

After evacuation of the mole she had reached the menopause. Three years later lung metastases and an elevated HCG titre (16 000 I.U./24 hrs) were found, but without local disease in the uterus. Combined treatment with MTX and Act. D was poorly tolerated and the interval between each course had to be longer than usual, up to 23 days. In spite of this the HCG level returned to normal after a total of 225 mg MTX and 5 mg Act. D. After one extra course of combined chemotherapy the patient was discharged. The HCG level at that time had been within the normal range for 6 weeks.

Six weeks later she was readmitted for check-up, and the HCG titre was found to be slightly elevated, 600 I.U./24 hrs. A chest X-ray disclosed new metastases. In spite of treatment with MTX, Act. D and vincaleucoblastine the patient ultimately died. The treatment during her second stay in the hospital was complicated by severe side effects, especially in the skin and the gastrointestinal tract.

Case 26 age 56, had 10 normal pregnancies. At an age of 54 she delivered a hydatidiform mole. Twenty-six months later CC with widespread metastases was found. Combined treatment with MTX and Act. D was started, but because of excessive bleeding hysterectomy had to be performed after one course of chemotherapy. In spite of intensive treatment with MTX, Act. D and ultimately vincaleucoblastine, the patient died 4 months later. Spread was found to lungs, liver, brain, kidney and suprarenal glands.

Case 29 age 52, had 10 normal pregnancies and one abortion 4 years before diagnosis of CC with metastases was made. During these 4 years she had no menstrual periods. The first symptom was vaginal bleeding which occurred 9 weeks before the diagnosis was made. Four weeks before admission she had hemoptyses followed by dyspnoea. The patient was extremely ill when she came into the hospital and died before the first course of chemotherapy was completed. At autopsy multiple, large, necrotic lung metastases and metastases to the vagina and pelvic lymph nodes were found. The primary tumour in the uterus measured approximately 14 cm in diameter.

Table I. *Preceding pregnancy and histological diagnosis*

Histology	Preceding pregnancy			
	Mole	Abortion	Term delivery	Total
Uncertain	7	—	—	7
Invasive mole	4	—	—	4
Choriocarcinoma	1	2	—	3
Metastatic choriocarcinoma	7	6	3	16
Total	19	8	3	30

Four cases without histological conformation of diagnosis.

more than 1 year after evacuation of a supposedly benign mole Table II shows the interval between the preceding pregnancy and diagnosis of MTN in relation to the histopathological diagnosis and the occurrence of metastases. It is evident that making an early diagnosis is as important in MTN as in other fields of oncology The table also illustrates the well-known fact that in some cases metastases may develop extremely rapidly in the course of the disease.

The age distribution appears in Fig. 1 The youngest patient was 17 and the oldest 58 years of age at the time of diagnosis. Four patients were postmenopausal with an interval between the last menstrual bleeding and the detection of MTN of from 1 to 4 years.

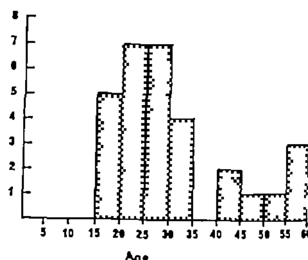


Fig 1 Age distribution. Three of the patients were postmenopausal.

METHODS

Monitoring of treatment was, in the earlier years of the study period, performed by determinations of HCG in urine with the haemagglutination inhibition method (13) Since 1968 radioimmunoassay of luteinizing hormone (LH) in serum (8, 9) has also been used.

In cases treated by chemotherapy alone the effect of treatment upon the local disease in the uterus was followed by repeated pelvic arteriograms. The usefulness of this procedure has been discussed earlier (5).

General principles of treatment which have developed in the present series have been as follows.

Table II *Time interval from preceding pregnancy to diagnosis*

Histology	Time interval in months					Total
	2	3–5	6–11	12 23	24–48	
Uncertain	5	2	—	—	—	7
Invasive mole	3	1	—	—	—	4
Choriocarcinoma	—	1	—	1	1	3
Metastatic choriocarcinoma	3	2	2	4	5	16
Total	11	6	2	5	6	30

Invasive mole localized to the uterus was usually treated with methotrexate (MTX) only Initially 25 mg was given in daily intramuscular injections for 3–5 days depending upon body weight, general condition and blood examinations. In an attempt to counteract the most unpleasant side effect which, in our experience, has been stomatitis, the patient was encouraged to rinse her mouth at frequent intervals for at least 1 hour immediately after each injection, with a mouthwash consisting of 3 mg citrovorum factor (Leucovorin) and 1 000 I.U hyaluronidase (Penetrase) dissolved in glass of water

If there was manifest metastasis from an invasive mole or a histologically proven choriocarcinoma, in addition to methotrexate 0.5 mg actinomycin D (Act. D) was given daily for 3–5 days. The drug was given intravenously in 1 000 ml saline over a period of at least 3 hours. The interval between each course of MTX or combined MTX/Act. D varied from 6 to 14 days depending upon toxicity symptoms and blood examinations. It was deemed desirable that the courses should follow one another as closely as possible, especially in cases with widespread metastases. The number of courses depended upon the effect of the treatment on the HCG or LH level in urine or serum. Gonadotrophin determinations were carried out each week during the treatment. A full blood count was done twice weekly differential count, and liver and kidney function tests once a week.

In patients with anemia blood transfusions were always given before treatment started As an adjuvant to chemotherapy and with the aim of stimulating the bone marrow and improving the general condition of the patients, 100 mg of an anabolic steroid, methenolone oenanthate (Primobolan®-Depot, Schering), was administered each week, and the patients were kept on diet rich in protein, minerals and vitamins.

With increasing experience with chemotherapy surgical treatment involving hysterectomy was considered advisable only for complications such as excessive bleeding and per

LH values were slightly elevated when treatment was stopped. Moreover definitely pathological LH titres were observed several weeks before the HCG values became abnormal.

Previously it has been mentioned that at least two or perhaps three courses of chemotherapy should be given after normal levels of HCG-LH have been reached. This is an arbitrary recommendation. The exact time when all tumour cells have been killed can only be guessed. Bagshawe (1) has calculated that a patient whose HCG-LH output is in the normal range, may still have several thousand active tumour cells, since 10 IU HCG may represent the production of 20 000 tumour cells with a total volume of about 0.1 mm³.

A prompt response to treatment with a rapid and constant fall of the HCG-LH level seems to indicate good prognosis. Fluctuating hormone titres during chemotherapy should be regarded with suspicion. In such cases it seems wise to administer more than three courses of chemotherapy after normal values have been reached. An illustration of this last type of response is shown in Fig. 2. The case history was as follows:

Case 11, age 25. The patient aborted in the third month of her first pregnancy. Eleven months later choriocarcinoma was diagnosed by arteriography and curettage. Response to chemotherapy with MTX at first seemed satisfactory but a new rise in the HCG-LH level led to combined treatment with MTX Act. D (Fig. 2). The patient received three combined courses after normal HCG level had been reached. In spite of this, a local recurrence in the uterus was detected 8 months later. Hysterectomy was performed under cover of chemotherapy and the patient has now been without signs of recurrence for more than 3 years.

Complications of chemotherapy

There have not been any fatal complications in the present series. A slight rise of temperature following one or more treatment courses was observed in 15 of 28 cases (Tables IV and V). Exanthema was seen in 5 cases, and in one of the patients the rash was severe.

The most troublesome complaint was stomatitis which we have tried to counteract by a mouthwash containing curovorum factor and hyaluronidase. It is difficult to evaluate whether this procedure has really been of any benefit. Four patients had severe stomatitis with ulcer formation, 11 patients had a slighter reaction and in 13 patients stomatitis did not develop. There was no direct correlation between the dosage of MTX and Act. D and the occurrence of stomatitis. The mucosal reaction was seldom so severe that it was necessary to postpone the next course of chemotherapy.

Table IV. *Complications of chemotherapy with Methotrexate alone*

Dose of MTX (mg)	Fever	Exanthema	Stomatitis	Alopecia	Pleurisy
75					
75					+
275	+		+ +		
275	+		+	+ +	
300	+		+ +	+ + +	
325				+ +	
325	+ +				
450			+ + +	+	
475					
475		+ +			
475			+ + +		
500		+	+		
575	+				+

Hair loss was seen in 16 patients, and more often when combined MTX/Act. D treatment was given than in cases receiving MTX only (cf. Tables IV and V). In no instance was the alopecia permanent. In the course of 3 to 6 months, all patients had a normal growth of usually thicker hair than before treatment.

Three patients complained of pleuritic pain, and in two of these a slight accumulation of fluid in the pleural cavity could be seen on chest X-ray films.

Reduction in white blood cells or thrombocytes never was so pronounced that it was necessary to stop treatment or postpone the next course of chemotherapy. The lowest observed white blood cell count was 1 200 and that of platelets 31 000. Routine use of the anabolic steroid methenolone oenanthate may have had some beneficial effect on the myelopoiesis, preventing a more severe depression of the bone marrow. Any definite proof of this is lacking, but there is clear evidence that the drug stimulates erythropoiesis, since the hemoglobin levels in almost all patients in the present series became higher than normal in women of the same age groups after 4 to 6 weeks of treatment.

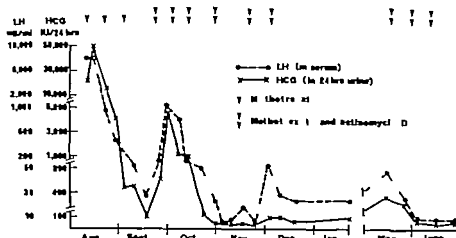


Fig 2 An example of fluctuating hormone levels during treatment with chemotherapy Local recurrence in the uterus was found 8 months after HCG in 24 hrs urine became normal. Note that LH in serum during these months was slightly elevated on several determinations when the HCG values were within the normal range.

In retrospect and in view of our present experience it may be justified to conclude that case 19 was not properly treated. Dissemination of tumour tissue probably occurred during manipulation of the uterus at the time of surgery After the operation the patient should have been closely followed until the HCG titre returned to normal. Immediate postoperative treatment with MTX and Act. D might possibly have cured the patient.

Cases 15 26 and 29 all were remarkable in the respect that the disease was detected several years after menopause. It is, however a well known fact that the latent period from the last known pregnancy to the occurrence of symptoms in some cases of MTN may be very long. Case 15 was difficult to treat because of poor tolerance to the drugs used. Cases 26 and 29 were too advanced when chemotherapy was started Although in this series as in other series reported in the literature patients with dissemination, e g to the lungs, vagina, kidney and brain have been cured, modern chemotherapy is not without failures.

Monitoring of treatment

Malignant trophoblastic neoplasia is unique in the respect that the tumour produces a specific index substance which makes it possible not only to establish the diagnosis, but also to evaluate the cellular response to therapeutic agents. The measurement of chorionic gonadotrophin in urine or serum has shown to be a reliable method of monitoring chemotherapy Pelvic arteriograms at certain intervals are helpful in the evaluation of the reduction in size of the uterine tumour It should be pointed out, however that the HCG-LH level is a more reliable index than are the arteriographic findings. The hormone level not only reflects the size of the primary tumour but also that of possible hidden metastases. Moreover there is a definite delay in the return of arteriographic findings to normal. The pathological vascular system induced by the tumour may persist for several weeks, in some cases for several months after all tumour tissue has been eradicated. In the present series, 2 out of 14 cases treated by chemotherapy alone developed persistent arterio-venous fistulae which were closed surgically 4 and 24 months respectively after completion of chemotherapy In 3 other cases definite pathological arteriograms were observed from 4 to 16 weeks after the hormone level returned to normal values and treatment stopped.

We agree with other authors that it is of the utmost importance to have a reliable and sensitive method for the determination of HCG Since the available assay systems do not distinguish between LH and HCG HCG production is inferred to be taking place when the excretion rate exceeds the normal range of LH. In the first years of the study period HCG in 24-hour urine specimens was determined by the haemagglutination inhibition test of Wide & Gemzell (13) Since 1963, LH in urine and serum has been determined by a radioimmunoassay method (8 9) Four times we have experienced that supposedly normal HCG titres within the range 20–150 I.U /24 hours, as estimated after concentration of the urine were followed by recurrence of the disease In three instances concomitant determinations of serum LH had been performed. Review of the data revealed that in two of the three cases the serum

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Table V *Complications of combined chemotherapy with methotrexate and Actinomycin D*

Dose in mg of						
MTX	Act. D	Fever	Exanthema	Stomatitis	Alopecia	Pleurisy
250	7.5	+ +			+	+
300	7.5	+		+ +	+ +	
400	7	+ +				
425	5	+ +		+ +	+ + +	
475	9.5				+ +	
500	1.5	+ +	+ + +	+ + +	+ +	
575	13	+ +	–	+ +	+ + +	
625	10				+ + +	
700	5			+		
700	9	+		+ +	+ + +	
800	16				+ +	
850	12	+	+ +	+ + +	+ + +	
1125	2					
1135	15.5			+ +	+ + +	
1225	12				+	

COMMENTS

The experience in the chemotherapy of malignant trophoblastic neoplasia here reported agrees well with that of other authors (1 2 4 6 12) The most important observation is that metastatic MTN is curable. Although the observation period for som patients is short it seems justified to presume that 12 out of 16 patients with metastases will be permanently cured. This is a very satisfactory result especially taking into account the case histories of those 4 not being cured.

In agreement with Bagshawe (1) Ross et al. (12) Lewis (6) and others, one of the most important prognostic criteria was found to be the interval from the antecedent pregnancy to the time of diagnosis. One of the four fatal cases was detected 17 months and the rest more than 24 months after the preceding pregnancy Making an early diagnosis is thus a vital problem. With the routine use of sensitive and reliable gonadotrophin assays it should at least be possible to detect at an early stage all cases of MTN developing after hydatidiform mole Radioimmunoassay of LH in serum has proved to be a more sensitive system than the hemagglutination inhibition test for determination of HCG in 24 hours urine.

An important observation is that a somewhat delayed response to chemotherapy with slightly fluctuating hormone titres during treatment may be of prognostic significance. This type of response has in three cases in the present series been followed by recurrence of the disease several months, in one case as long as 13 months, after gonadothrophin levels within the normal range had been reached. Bagshawe (1) has also stressed that th fall and rise of HCG excretion during therapy should indicate more intense and prolonged treatment.

In an earlier paper (5) it has been suggested that pelvic arteriography is of great value in the early diagnosis and also in the management of malignant trophoblastic neoplasia. If for example the HCG-LH titre after delivery of a hydatidiform mole has not returned to normal in the course of 4–6 weeks, angiography may disclose residual tumour in the uterine wall which may not be detectable by curettage. In our opinion it is correct in such cases to start chemotherapy even if histological confirmation of the diagnosis has not been obtained. In the follow-up of MTN with arteriography it should be remembered that the pathological vascularization induced by an invasive mole or a choriocarcinoma may persist for a relatively long period after all tumour tissue has been eradicated and the hormone level has returned to normal An interval from 4 to 6 weeks has been observed in the present series, and in two cases persistent arteriovenous shunts were found.

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I the histopathological classification of the cases in the present series had the pri ilege t have the expert assistance of Wallac W Park, The University Dundee, Scotland W gratefully acknowledge this he p

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FIBRINOLYTIC ACTIVITY OF VEINS DURING TREATMENT WITH MEDROXYPROGESTERONE ACETATE

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Abstract. Coagulation and fibrinolytic studies were performed on 18 postmenopausal women receiving medroxyprogesterone acetate 5 mg a day by mouth for one month. The fibrinolytic activity in the vessel wall as determined histochemically in vein biopsy specimens and the blood after stimulation by venous stasis.

In contrast with what is seen after oestrogen treatment the fibrinolytic activity in the vein vessel wall was unchanged. Neither were any changes found in the response of the fibrinolytic activity to venous stasis, or of the coagulation factors or components of the fibrinolytic system. The investigation thus produced no evidence of thrombogenic effect of medroxyprogesterone acetate.

The introduction of contraceptives containing progestogens alone soon roused interest in their possible effect on coagulation and fibrinolysis.

The progestogens that have received most attention are chlormadinone acetate and medroxyprogesterone acetate. The former preparation has been found to accelerate platelet aggregation (20) but not to have any effect on platelet adhesiveness (15). The use of progestogens has been proved not to alter any coagulation factors (4, 8, 15). Nor have they been found to produce any changes in the plasminogen, alpha$_2$-macroglobulin or inhibitors of urokinase-induced fibrinolysis (4, 7, 8, 12, 15). They have, however, been shown to cause a moderate depression of the fibrinolytic activity in the blood (4, 13).

The fibrinolytic activity in the vein wall is of particular interest in the light of Isacson & Nilsson (10) finding of a low fibrinolytic activity of the veins in 55% of patients with recurrent idiopathic thrombosis and the observation by Pandolfi et al. (18) of a lower activity in lower leg veins than in arm veins, a phenomenon in accord with the higher frequency of thrombosis in the lower leg than in the arm.

In previous studies the fibrinolytic activity in the vein wall was unchanged in women who had been receiving chlormadinone acetate continuously (15) and in women examined after about one year's use of medroxyprogesterone acetate 150 mg parenterally every third month (27).

In the present investigation the dose used was larger and the patients served as their own controls. Medroxyprogesterone acetate was studied for its effect on the fibrinolytic activity in the vein wall and on the local blood fibrinolytic activity after venous occlusion of the arms. The investigation was extended to determine some of the coagulation factors and some other components of the fibrinolytic system.

MATERIAL AND METHODS

The clinical material consisted of 18 postmenopausal women, aged 54 to 77, who were to undergo operation for uterine prolapse, but who otherwise felt well at the time of the investigation. They were receiving medroxyprogesterone acetate in a dose of 5 mg a day by mouth for one month. Blood samples and vein biopsy specimens were obtained before and on the last day of treatment.

Coagulation studies. The following determinations were made: fibrinogen (blood collected with EACA), prothrombin + factor VII + factor X (Owren P&P-test), factor V and factor VIII. The methods described previously (14) were used for the determinations.

Fibrinolytic studies. The following determinations were made: fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates expressed as mm² of lysis, plasminogen (immunological method), inhibitors of plasminogen activation by urokinase (urokinase inhibitors), alpha$_2$-macroglobulin (esterolytic meth-

…gnosis was not certain. In a large series of …men using chlormadinone acetate Butler & …l (5) found only one case of phlebothrombosis, …d then in a women who had had a similar …isode during pregnancy

In the present study in which the dose of …droxyprogesterone acetate was large and in …ich the patients served as their own controls, … changes were found in the coagulation or …inolytic systems. The local response of the …rinolytic activity to venous occlusion tended to …crease, but not significantly The fibrinolytic …tivity in the vein wall did not change.

The absence of incriminating data in the litera…re together with the lack of any demonstrable …fect of medroxyprogesterone acetate on the …rinolytic activity in the vein wall in the present …aterial corroborate the assumption that proges…gens have no appreciable thrombogenic effect.

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Table I. *Fibrinolytic activity of hand veins before and after use of medroxyprogesterone acetate for one month (histochemically determined. Arbitrary units)*

Treatment	Range	Median value
Before	4.5–10	7
After	3–10	7

Table II *Mean values for fibrinolytic components before and after use of medroxyprogesterone acetate for one month*

Component	Before	After
Plasminogen, %	112±23	104±14
Urokinase inhibitors, %	122±19	120±25
Alpha$_2$-macroglobulin, %	128±24	128±28
Spontaneous fibrinolytic activity on unheated f brin plates		
Resusp. euglob prec., mm^2	19±21	21±26
Fibrinolytic activity on unheated fibrin plates during venous occlusion		
Resusp euglob. prec., mm^2	303±185	278±146

od) and fibrinogen-fibrin degradation products (FDP) (immunochemical method, blood collected with EACA). This method will not demonstrate FDP in normals i.e. <5 μg. The methods are described previously (14).

Venous stasis (22) was produced by placing sphygmomanometer cuff around the upper arm and inflating it to a pressure midway between the systolic and diastolic pressure, f r 20 min. Blood samples for the determination f the fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates (1, 16) were drawn from an antecubital vein before the cuff was inflated (spontaneous fibrinolytic activity) and again immediately before it was deflated.

Venous biopsy specimens, about 0.5 cm long, were obtained from the hand under local anaesthesia. The fibrinolytic activity was demonstrated with the histochemical method of Todd (25), as modified and graded ccording to Pandolfi t al. (19).

The portions of vein were cut on an International Harris cryostat in sections 8 μ thick and collected on pre-cleaned glass slides. Four slides were prepared for each sample. The sections on each slide were covered with

Table III *Mean coagulation values before and after use of medroxyprogesterone acetate for one month*

Factor	Before	After
Fibrinogen, g/100 ml	0.36±0 09	0.37±0.09
P & P (factor II VII, X), %	106 ±10	111 ±15
F ctor V %	111 ±13	105 ±15
Factor VIII, %	136 ±32	126 ±42

0.06 ml of fibrinogen (bovine fibrinogen essentially prepared according to Brakman (3) in concentration of 1% in phosphate buffer pH 7.8, ionic strength 0.15) and of 10 μl thrombin (Topostasin 20 NIH units/ml unbuffered saline). The fibrinogen-thrombin mixture was spread over an area of 10 cm^2 in order to obtain a fibrin film about 0.07 mm thick. To stabilize the fibrin film, the slides were left at room temperature (21 24°C) in a moist chamber for 30 min. One of the slides was fixed in formalin immediately afterwards while the remaining 3 slides were transferred to another moist chamber at 37°C and incubated for 10, 20, 30 min, respectively after which they were fixed in formalin. The fibrin slides and the sections were stained with Harris' haematoxylin. Fibrinolysis was reflected by clear lytic areas in the fibrin film at the site of fibrinolytically active cells. Three fairly distinct grades of fibrin digestion were recognized, namely *grade I* microscopical punctate areas in most of the sections, *grade II* gross lytic areas of irregular outline and sometimes confluent *grade III* dissolution of most or all the fibrin in contact with the sections.

A grade I slide was allotted 1 point; a grade II slide, 2 points, and a grade III slide, 3 points. The total number of points scored by the set of 4 slides was taken as a measure of the fibrinolytic activity of the sample.

Statistical methods. The individual groups were compared by Student s t-test. The difference in the fibrinolytic activity of the veins was analyzed by the Wilcoxon rank sum test.

RESULTS

The fibrinolytic activity in the wall of the hand veins was unchanged (Table I) The local response of the fibrinolytic activity to venous occlusion of the arms was 303 ± 185 mm^2 before treatment and 278 ± 146 after treatment. The difference was not significant ($p > 0.05$) (Table II) No changes were found in the plasminogen, urokinase inhibitors and alpha$_2$-macroglobulin (Table II) or in the coagulation factors studied (Table III) No FDP were found in any of the patients.

DISCUSSION

The oestrogenic preparation ethinyloestradiol has been found to lower the fibrinolytic activity of the vessel wall (26) The oestrogenic component in combined oral contraceptives (9) and treatment with oestrogens alone (2, 6, 11 17) have been found to increase the frequency of thrombotic complications.

There is no evidence of a correlation between the use of progestogens and the frequency of thrombosis (23 24) Powell & Seymour (21) followed up 1 123 patients using medroxyprogesterone acetate and found only one case of deep venous thrombosis, and even in that case the

...sis was not certain. In a large series of ...men using chlormadinone acetate Butler & ...ll (5) found only one case of phlebothrombosis, ...d then in a women who had had a similar ...isode during pregnancy

In the present study in which the dose of ...edroxyprogesterone acetate was large and in ...ich the patients served as their own controls, ...o changes were found in the coagulation or ...brinolytic systems. The local response of the ...brinolytic activity to venous occlusion tended to ...crease, but not significantly. The fibrinolytic ...ctivity in the vein wall did not change.

The absence of incriminating data in the litera...ure together with the lack of any demonstrable ...ffect of medroxyprogesterone acetate on the ...ibrinolytic activity in the vein wall in the present ...aterial corroborate the assumption that progesta...ogens have no appreciable thrombogenic effect.

ACKNOWLEDGEMENT

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Adenocarcinoma Registry

Clear-cell adenocarcinomas of the genital tract (mesonephromas) in young women have recently been associated with maternal stilbestrol administration (N Engl J Med *284* 878 1971 Cancer *25* 745 1970). Since the experience of any one clinic with these tumors will be small, a Registry supported by grants from the American Cancer Society and National Institutes of Health has been established to centralize data and to study pathogenesis, maternal histories, and therapy from cases in the United States and abroad.

Confidentiality will be strictly maintained. No patient will be identified in any information or report released from the Registry Data is sought on all cases *including those to be published by the reporting physician or institution* Specific permission will be obtained to include the information from each case in any reports emanating from the Registry

We seek information on all cases of clear-cell adenocarcinoma of the genital tract in women under age 25 years *whether or not there is a history of maternal hormone ingestion.* Please direct all communications to

Registry for Clear-Cell Adenocarcinoma (Mesonephroma) of the Genital Tract in Young Women
Warren 275
275 Charles Street
Boston, Mass. 02114
USA

Arthur L. Herbst, M.D. Director

Robert E. Scully M.D., Pathologist

German Society of Endocrinology

The German Society of Endocrinology has granted the Schoeller Junkmann-Award 1972, donated by Schering AG for three papers.

Dr Rüdiger K. Wagner Max Planck Institut für Zellbiologie 294 Wilhelmshaven, was awarded the first prize (DM 7 000 —) for his paper Prinzip und Anwendung der Mikrobestimmung von Steroidhormonrezeptoren durch Agargel-Elektrophorese

Dr Gerhard Leyendecker Universitäts-Frauenklinik Bonn, 53 Bonn-Venusberg, was awarded the second prize (DM 5 000 —) for his paper "Studies on the endocrine regulation during the periovulatory phase of the human menstrual cycle the effects of oestradiol-17β and progesterone on the release of pituitary LH and FSH"

Dr Matti J Tikkanen and Prof Dr Herman Adlercreutz, Dept. of Clinical Chemistry University of Helsinki, Finnland were awarded a further second prize (DM 5 000 —) for their paper Urinary estriol conjugates in pregnancy Studies on the urinary excretion of estriol conjugates in normal pregnancy and in recurrent intrahepatic cholestasis of pregnancy"

The "Marius-Tausk Förderpreis" donated by Organon GmbH München was not awarded this year

Acta Obstet Gynec Scand 51 287–292, 1972

EVALUATION OF A CAPILLARY TUBE SPERM PENETRATION METHOD FOR FERTILITY INVESTIGATIONS

Magnar Ulstein

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Abstract. The sperm penetration test introduced by Kremer was studied, using cervical mucus as the test medium and human semen samples with varying properties. The spermatozoa from semen samples of good quality penetrated rapidly to more than 30 mm within one hour. Spermatozoa from semen samples of poor quality penetrated slowly and a maximum penetration of less than 20 mm was noted. Capillary tube size, incubation temperature, and the amount of mucus protruding from the capillary tube, had no appreciable influence on the results. Reproducibility was good. Storage of the cervical mucus at +4°C or −20°C for 3 weeks did not influence its suitability as test medium. The test is easy to perform and well suited for routine semen analysis.

The ability of spermatozoa to penetrate cervical mucus can be tested in vivo by postcoital testing. However in vitro methods have been developed using slides (2, 8, 11) or capillary tubes (3, 5, 6, 9). Botella-Llusia introduced in 1956 a capillary tube method where a drop of semen was placed on a hollow-ground glass slide, and one end of a capillary tube filled with test medium was immersed in the semen.

In 1965 Kremer modified the capillary tube method, using cervical mucus or serum as the test medium. The distance which the foremost spermatozoa had penetrated was taken as a measure of linear penetration. Most of the capillary methods require relatively large volumes of test medium, for Kremer's method about 50 µl and for that of Botella-Llusia (5) 35 µl. Carlborg (6), however used very small quantities of cervical mucus and read the tests after 5 min.

The capillary tube sperm penetration test is found to be valuable in fertility investigations, but further evaluation of the method using cervical mucus as the test medium is required before the test is included in routine semen analysis.

The aim of the present investigation was to evaluate the reliability and variable factors of the capillary tube test and arrive at a suitable combination to be employed in a routine method for testing sperm penetration of cervical mucus.

MATERIAL AND METHODS

The penetration tests were performed according to the method of Kremer (9) with a sperm penetration meter consisting of a calibrated glass slide to which a support and a reservoir are fixed (Fig. 1). Semen was pipetted into the reservoir, and one end of a capillary tube containing cervical mucus immersed into the semen, the cervical mucus protruding slightly from the end of the capillary tube. The other end was sealed and supported by modelling clay. The setup was placed horizontally in a Petri dish containing a piece of moist filter paper and incubated at 37°C, unless otherwise stated. Readings were made with a microscope, and the penetration of the foremost spermatozoon recorded in mm.

The cervical mucus was collected from the cervical canal of gynecologically healthy women at the expected time of ovulation. The portio was first cleaned with dry gauze and a small ring-forceps inserted into the cervical canal, opened and withdrawn half-closed. Special precautions were taken not to contaminate the cervical mucus with vaginal content. To be accepted as test medium the mucus was required to fulfill the following criteria: clear without clumps or turbid areas; low viscosity and easily drawn into small capillary tubes; "spinnbarkeit" of at least 10 mm; and a strongly positive fern test. Finally the cervical mucus samples were tested with a semen sample from one fertile donor with known sperm penetration ability and a penetration of at least 40 mm in 1 hour was required. For parts of the investigation large volumes of mucus were needed. These samples were taken from one woman 3 or 4 times at the same days of the cycle. The capillary tubes filled with cervical mucus were sealed and stored at +4°C or at −20°C prior to use. When possible, the experiments were done with fresh cervical mucus.

The semen samples analysed were donated by 16 men,

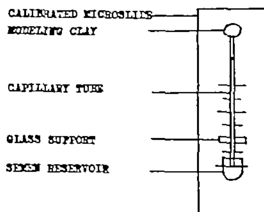


Fig 1 Sperm penetration meter and capillary tub filled with cervical mucus.

some known to be fertile (nos. I–IX), and the others (nos. X–XVI) from the infertile clientele of the clinic. The samples were taken by masturbation after t least 3 days of continence and brought to the laboratory in a plastic condom, specially designed for this purpose. Analyses were started within hours of ejaculation and were performed according to the routine of the laboratory which includes specification of volume sperm density percentage of motile spermatozoa, qualitative motility (classified from 0 to 4), percentage of abnormal spermatozoa, percentage of living spermatozoa, presence of leucocytes or other cells, and content of fructose and acid phosphatase in the seminal plasma. Table I presents the mean values for the tested semen samples from the 16 donors.

EXPERIMENTS AND RESULTS

Influence of the time

Capillary tubes with an internal diameter of 1 0 mm and a length of 67 mm were used. The cervical mucus column in the capillary tubes was about 65 mm long. Fig. 2 shows the means for sperm penetration observed during 4 hours in triplicate tests of semen samples from 16 donors. For semen samples of good quality the penetration was considerable after half an hour and was almost linear during the first hour For semen samples of decreased quality the penetration at half an hour was small, and the differences between the samples were small. Linear progression persisted for about 3 hours, at which time more pronounced individual differences were noted. The ranges for the 3 tests of each sample were greatest at the half-hour readings and for semen with high penetration, the maximum being 21 mm for semen from donor II. For all semen samples the range decreased with time and at 3 hours the maximum range was 4 mm. The highest observed sperm penetration was 63 mm in 30 min, that is _1 mm/min.

Influence of the si e of the capillary tube

Capillary tubes with internal diameters of 0.6, 0 8 and 1 0 mm and with lengths of 41 40 and 67 mm, respectively were used. Semen from donor VIII was tested in 3 capillary tubes of each size filled with cervical mucus from one specimen. The results are shown in Table II. The sperm penetration showed only slight differences in relation to the size of the capillary tube, larger ranges of penetration being noted for the larger tubes in the early readings. At 3 hours, however the means and ranges were very similar

Table I *Properties of all tested semen samples given as mean values for each donor*

Donor	Volume (ml)	Sperm density (mill./ml)	Living ( )	Motile (%)	Motility degree	Abnormal forms ( )
I	5.5	150	78	65	4	40
II	[illegible]	107	[illegible]	60	3	47
III	3.8	19	83	60	3	3
IV	2.6	[illegible]	77	65	3	[illegible]
V	4 6	115	74	60	4	35
VI	[illegible]	146	69	55	3	[illegible]
VII	3.8	190	73	55	3	34
VIII	4.1	181	81	60	3	31
IX	3 1	60	62	40		68
X	4.2	33	53	30	3	51
XI	3.5	15	65	45	3	56
XII	1 7	18	51	35		32
XIII	[illegible]	3	47	[illegible]		40
XIV	[illegible]	15	38	20		48
XV	6.1	45	4	20	1	68
XVI	[illegible]	10	39	15		78

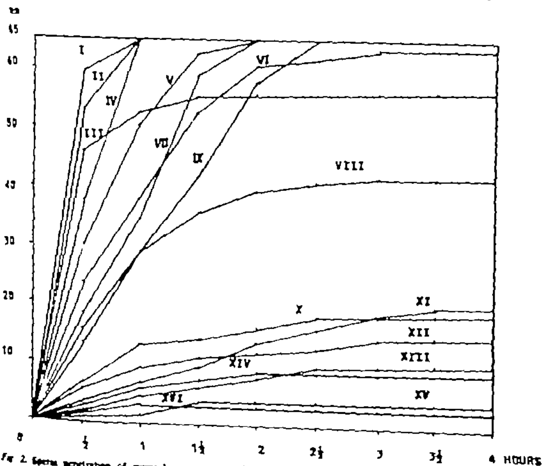


Fig. 2. Sperm penetration of cervical mucus in relation to time. Means of triplicate tests of ejaculates from 16 donors.

Influence of the ambient temperature

These tests were performed in triplicate at 22 C and at 37 C. Semen from donor III and cervical mucus from one specimen in capillary tubes with internal diameter 1.0 mm were used. Table III shows the results. The sperm penetration was greater at 37 C than at 22 C, but the differences were very small.

Influence of the amount of mucus protruding from the capillary tube

The size of a mucus drop protruding from a capillary tube is very difficult to measure. Three estimations were made: Mucus at the end but not protruding from the capillary tube; mucus protruding about 1.5 mm, and about 2 mm. Capillary tubes with internal diameter of 1.0 mm were

Table II. *Effect of size of capillary tube on sperm penetration (expressed in mm). T sts in triplicate ith semen from donor VIII*

Tube dimensions (mm)	1/2 hour		1 hour		2 hours		3 hours	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
0.6 41	18.2	7	27.9	7	38.0	5	38.3	3
0.8 40	17.4	11	28.3	9	37.1	7	37.4	4
1.0 37	15.2	12	29.0	10	39.2	6	40.5	4

Table III. *Effect of incubation temperature on sperm penetration (expressed in mm). Tests in triplicate with semen from donor III*

	1/2 hour		1 hour		2 hours		3 hours	
Temperature	Mean	Range	Mean	Range	Mean	Range	Mean	Range
22°C	43 1	7	50.3	11	55.1	7	55.1	3
37°C	46.2	11	52.5	9	55.2	10	56.0	4

Table IV *Effect of size of mucus drop on sperm penetration (expressed in mm) Approximate number of spermatozoa per visual field (magnification 100 ×) Tests in triplicate Donor IX*

	1/2 hour		1 hour		2 hours		3 hours	
Protrusion of mucus from tube (mm)	Mean	Sperms *n*	Mean	Sperms *n*	Mean	Sperms *n*	Mean	Sperms *n*
2.0	15	20–30	29	30–40	60	30–40	65	30–40
1.5	13	15–20	28	20–25	58	35–40	59	35–40
0	17	20–30	29	25–30	60	25–30	65	30–35

used and all tests were done in triplicate with semen from donor IX and cervical mucus from one batch. In this experiment the spermatozoa in the visual field including the foremost spermatozoa at magnification 100 × were counted.

The results are shown in Table IV There were only small differences in sperm penetration and numbers of spermatozoa per visual field in relation to the different amounts of mucus protruding from the capillary tube.

Reproducibility

The number of replicate tests with semen from each donor was limited by the amount of cervical mucus in the mucus batches. Capillary tubes of 1 0 mm internal diameter were used. 11 with a semen sample from donor III 8 with a semen sample from donor V and 10 with a semen sample from donor VI

Table V shows that the ranges and standard deviations for sperm penetration were small and decreased in the late readings.

Reproducibility was also tested by using different ejaculates from the same donor and testing them in tubes of the same batches of cervical mucus from 3 women. The tubes of mucus were kept at 4 C until used. Fresh ejaculates from donors III, VIII, XIII and XV were tested once or twice a week.

It can be seen from Table VI that the sperm penetration of different ejaculates from the same donor did not vary greatly and the penetration of spermatozoa from the same ejaculate through cervical mucus from different women also showed only small variations.

Influence of storage on cervical mucus

For this experiment 60 capillary tubes of cervical mucus taken from the same woman were used Thirty of these were stored at +4 C and the

Table V *Sperm penetration (in mm) in several tubes of one batch of cervical mucus*

S D = standard deviation

		1/2 hour			1 hour			2 hours			3 hours		
Donor	No. of tubes	Mean	Range	S.D	Mean	Range	S.D	Mean	Range	S.D	Mean	Range	S.D.
III	10	47 1	17	7.2	51 7	11	4.7	54.2	1	5 1	54 3	5	2.1
V	8	51.2	17	6.1	51.2	9	4.3	64.3	13	5.5	64.7	7	3.2
VI	11	44.1	1	7.5	47.5	14	5 1	60.9	12	4.2	63.9	6	2.3

Table VI. Sperm penetration (in mm). Tests with several ejaculates from 4 donors tested in cervical mucus from 3 women

Donor	Ejaculate	Mucus A	Mucus B	Mucus C	Range
III		39	51	55	8
	b	51	49	52	3
		56	57	51	6
	d	53	49	57	8
Range		8	8	6	
VIII	a	39	40	39	1
	b	38	37	37	1
		37	33	33	4
	d	39	40	42	3
Range		2	3	9	
XIII		9	4	5	5
	b	7	10	9	3
		6	7	5	2
Range		3	6	4	
XV		4	5	3	2
	b	7	4	3	4
		3	5	3	2
Range		4	1	0	

ent at −20 C. Fresh ejaculates from donors III and VIII were tested once or twice a week in 3 capillary tubes which had been stored at +4 C and in 3 stored at −20 C. One reading was made at 3 hours.

Table VII shows the results. For cervical mucus stored at +4 C the penetrability was unchanged by storage up to 3 weeks. There was reduced penetrability with longer storage. Phalanx formations were seen. For cervical mucus stored at 20 C there was unchanged penetrability during the 5-week period, but after thawing, small air bubbles were observed in the mucus. These disturbed the readings. The ranges of penetration ere larger in tests using *frozen cervical mucus* n when the refrigerated samples were em- yed

DISCUSSION

When post-coital analysis shows poor penetration, it is difficult to say whether the fault lies with the quality of the semen or the cervical mucus. In these cases in vitro sperm penetration tests are of value. The capillary tube test is preferable because slide tests do not permit an exact measurement of linear penetration, and pseudopenetration may be noted when the drop of semen and the drop of cervical mucus flow out in such a way that they are superimposed.

Varying penetration times can be used. Kremer (9 10) read tests at half an hour and 2 hours. Carlborg (6) used a penetration time of 5 min and found a good correlation with measurements made at half an hour. The present investigation shows that spermatozoa from semen samples of good quality penetrate quickly to more than 30 mm. Spermatozoa from semen samples of poor quality penetrate slowly and 1 to 3 hours are required for maximum penetration which in this investigation did not exceed 20 mm.

In the present investigation the highest speed at which the spermatozoa penetrated was 2.1 mm/min. Kremer (10) recorded a maximum sperm penetration rate of 2.8 mm/min. These data are a little lower than those observed in vivo (1 4 12).

Neither the size of the capillary tube nor the ambient temperature should influence the reliability of the test. The size of the mucus drop protruding from the end of the capillary tube did not influence the results, but when performing the test it is of importance that there is a good contact between the semen and the cervical mucus and that no air bubbles appear in the cervical mucus column.

In the present investigation good reproducibility was noted when the same semen sample was tested with cervical mucus from different women.

ble VII *Sperm penetration (mm) through cervical mucus stored at* 4°C or 20°C Tests in triplicate

nor	Storage temp	1 week		2 weeks		3 weeks		4 weeks		5 weeks	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
	4°C	35	8	37	7	44	8	40	7	25	11
	20°C	49	11	50	13	55	14	58	9	46	9
II	4°C	42	7	44	9	48	11	32	4	31	10
	20°C	44	9	40	14	41	17	43	14	44	15

Table III. *Effect of incubation temperature on sperm penetration (expressed in mm). Tests in triplicate with semen from donor III*

Temperature	1/2 hour Mean	1/2 hour Range	1 hour Mean	1 hour Range	2 hours Mean	2 hours Range	3 hours Mean	3 hours Range
22°C	43 1	7	50.3	11	55.1	7	55.1	3
37°C	46.2	11	52.5	9	55.2	10	56.0	4

Table IV *Effect of size of mucus drop on sperm penetration (expressed in mm). Approximate number of spermatozoa per visual field (magnification 100 ×) Tests in triplicate Donor IX*

Protrusion of mucus from tube (mm)	1/2 hour Mean	1/2 hour Sperms n	1 hour Mean	1 hour Sperms	2 hours Mean	2 hours Sperms n	3 hours Mean	3 hours Sperms
2.0	15	20–30	29	30–40	60	30–40	65	30–40
1.5	13	15–20	28	20–25	58	35–40	59	35–40
0	17	20–30	29	25–30	60	25–30	65	30–35

used and all tests were done in triplicate with semen from donor IX and cervical mucus from one batch. In this experiment the spermatozoa in the visual field including the foremost spermatozoa at magnification 100 × were counted.

The results are shown in Table IV There were only small differences in sperm penetration and numbers of spermatozoa per visual field in relation to the different amounts of mucus protruding from the capillary tube.

Reproducibility

The number of replicate tests with semen from each donor was limited by the amount of cervical mucus in the mucus batches. Capillary tubes of 1 0 mm internal diameter were used 11 with a semen sample from donor III 8 with a semen sample from donor V and 10 with a semen sample from donor VI.

Table V shows that the ranges and standard deviations for sperm penetration were small and decreased in the late readings.

Reproducibility was also tested by using different ejaculates from the same donor and testing them in tubes of the same batches of cervical mucus from 3 women. The tubes of mucus were kept at 4 C until used. Fresh ejaculates from donors III, VIII, XIII and XV were tested once or twice a week.

It can be seen from Table VI that the sperm penetration of different ejaculates from the same donor did not vary greatly and the penetration of spermatozoa from the same ejaculate through cervical mucus from different women also showed only small variations.

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For this experiment 60 capillary tubes of cervical mucus taken from the same woman were used Thirty of these were stored at +4 C and the

Table V *Sperm penetration (in mm) in several tubes of one batch of cervical mucus*

S.D = standard deviation

Donor	No. of tubes	1/2 hour Mean	1/2 hour Range	1/2 hour S.D	1 hour Mean	1 hour Range	1 hour S.D	2 hours Mean	2 hours Range	2 hours S.D	3 hours Mean	3 hours Range	3 hours S.D
III	10	47 1	17	7.2	51 7	11	4.7	54.2	12	5 1	54.3	5	2.1
V	8	31.2	17	6.1	51.2	9	4.3	64.3	13	5.5	64 7	7	3.2
VI	11	24 1	21	7.5	47.5	14	5.1	60.9	12	4.2	63.9	6	2.3

Table VI. *Sperm penetration (in mm). Tests with several ejaculates from 4 donors tested in cervical mucus from 3 women*

Donor	Ejaculate	Mucus A	Mucus B	Mucus C	Range
III		59	51	55	8
	b	51	49	52	3
		56	57	51	6
	d	53	49	57	8
Range		8	8	6	
VIII		39	40	39	1
	b	38	37	37	1
	c	37	35	33	4
	d	39	40	42	3
Range		2	5	9	
XIII		9	4	5	5
	b	7	10	9	3
		6	7	5	2
Range		3	6	4	
XV		4	5	3	2
	b	7	4	3	4
		3	5	3	2
Range		4	1	0	

rest at −20°C. Fresh ejaculates from donors III and VIII were tested once or twice a week in 3 capillary tubes which had been stored at +4 C and in 3 stored at −20°C. One reading was made at 3 hours.

Table VII shows the results. For cervical mucus stored at +4 C the penetrability was unchanged by storage up to 3 weeks. There was reduced penetrability with longer storage. Phalanx formations were seen. For cervical mucus stored at −20 C there was unchanged penetrability during the 5-week period, but after thawing, small air bubbles were observed in the mucus. These disturbed the readings. The ranges of penetration were larger in tests using frozen cervical mucus than when the refrigerated samples were employed.

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When post-coital analysis shows poor penetration it is difficult to say whether the fault lies with the quality of the semen or the cervical mucus. In these cases in vitro sperm penetration tests are of value. The capillary tube test is preferable because slide tests do not permit an exact measurement of linear penetration, and pseudopenetration may be noted when the drop of semen and the drop of cervical mucus flow out in such a way that they are superimposed.

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In the present investigation the highest speed at which the spermatozoa penetrated was 2.1 mm/min. Kremer (10) recorded a maximum sperm penetration rate of 2.8 mm/min. These data are a little lower than those observed in vivo (1, 4, 12).

Neither the size of the capillary tube nor the ambient temperature should influence the reliability of the test. The size of the mucus drop protruding from the end of the capillary tube did not influence the results, but when performing the test it is of importance that there is a good contact between the semen and the cervical mucus and that no air bubbles appear in the cervical mucus column.

In the present investigation good reproducibility was noted when the same semen sample was tested with cervical mucus from different women.

Table VII. *Sperm penetration (mm) through cervical mucus stored at 4°C or 20°C. Tests in triplicate*

Donor	Storage temp	1 week		2 weeks		3 weeks		4 weeks		5 weeks	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
III	4°C	35	8	37	7	46	8	40	7	25	11
	20°C	49	11	30	13	55	14	58	9	46	9
VIII	4°C	43	7	44	9	48	11	32	4	31	10
	20°C	44	9	40	14	41	17	43	14	44	15

The range and standard deviation were small when several capillary tubes from the same cervical mucus sample were used for testing the same semen sample, and different ejaculates from the same donor had an almost constant penetration extent.

The reproducibility of the test was also good when cervical mucus stored for 3 weeks at +4 C was used, but longer storage time did change the penetrability. Freeze-preserved at −20 C the penetrability of the cervical mucus could be unchanged over longer time periods, but when thawed small air bubbles, which disturbed the readings, appeared in the mucus columns.

Clear and strict criteria were kept for the samples of cervical mucus to be accepted as test medium. The investigation does not concern cervical mucus of varying quality. Carlborg (6) stated that the penetrability of cervical mucus is highest the day prior to ovulation. The first day thereafter a strongly reduced penetrability was noted. Therefore careful testing of the cervical mucus samples is necessary. By keeping a constant quality of the cervical mucus used as test medium the reliability of the test is good. All cervical mucus samples should be tested with a semen of known penetration ability.

Investigating a group of men autoimmunized to spermatozoa, Fjällbrant (7) found that a penetration extent of less than 6 mm/3 hours was combined with infertility. He found a close correlation between sperm antibody titres and penetration extent. Botella-Llusia (5) found in a small series that spermatozoa from semen samples of definitely infertile men did not penetrate at all, while those from men known to be fertile penetrated 20.9 mm in half an hour. Further investigations *correlating fertility with the results of in vitro* sperm penetration tests were not found in the literature.

From the present investigation it can be concluded that the capillary sperm penetration test is reliable and reproducible when cervical mucus fulfilling certain criteria is used as test medium. In this only work an evaluation of the method was performed. The method offers the possibility of further investigations concerning correlation between *semen properties and penetration* and between penetration and fertility.

The technique of the test is very simple. No elaborate equipment is needed. The glass device described by Kremer *can easily be made*. Reading with the microscope is not difficult since the spermatozoa usually form a clear boundary. For practical use the method is simple, cheap, and can easily be performed in connection with routine semen analysis.

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POTENTIAL DIABETES IN WOMEN WITH LARGE BABIES

A Follow-up Study

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Abstract. This investigation is a follow-up of a previous study in which intravenous glucose tolerance tests (IVGTT) had been performed in women with complicated pregnancies. The series included 129 women with infants weighing 4.5 kg or more (LB) and a control group of 82 women with normal pregnancies and infants of normal weight (series I). In the present follow-up (series II), 82 of the women with LB and 52 of the control women could be retested. The time lapse between the testings was 5–7 years. In the control series, there was a significant mean decrease in K-values (K being the disappearance rate constant for intravenously injected glucose) from 2.12 ± 0.71 to 1.65 ± 0.59 S.D. In the group of women with LB the mean K-value decreased from 2.09 ± 0.77 to 1.35 ± 0.51 S.D. ($p < 0.001$). In the control group there were 7% and in the group of women with LB there were 4% who had K-values below the normal lower limit of 1.0. In series I the corresponding figures were 0% and 10%. 18% of the women with LB were overweight themselves and had a significantly lower K-value than the women with LB who were themselves of normal weight. The frequency of diabetes mellitus among first degree relatives of the mothers with LB was 28% and of the controls 2%. Between the two tests a further 2 women in this group had developed manifest diabetes compared to none in the control group.

Certain maternal and fetal complications can be used as indicators of a possible diabetic trait in the mother and for earlier diagnosis of diabetes mellitus (DM) (8). According to the recommendations of WHO (18) a woman who has given birth to a child weighing 4.5 kg or more is to be regarded as a potential diabetic. Several investigators have performed oral glucose tolerance tests (OGTT) in women 1–25 years after they had been delivered of a baby above 4.5 kg (4, 5, 6, 11, 12, 16) and impaired glucose tolerance had developed in frequencies from 10–50%.

Intravenous glucose tolerance tests (IVGTT) were performed in a series of women with previously complicated pregnancies (8) including those who had given birth to infants above 4.5 kg (LB). The mean rate constant for the disappearance of intravenously injected glucose from the blood (expressed as $100\,k = K$) was found to be significantly lower in women with LB ($p < 0.001$) as compared with that of women with normal obstetrical records and with infants of normal birth weight. Among the women with LB 10% had a reduced IVGTT defined as a K-value below 1.0 as compared with none in the control group when tested 3–32 years after the delivery.

As it is of great interest to know about the future development of a diabetic state in the material studied, a follow-up investigation was made. The present study deals with the result of IVGTT performed in the women with LB and in the control group after an additional time lapse of 5–7 years from the first test.

MATERIAL AND METHODS

In the material published in 1966 (series I) there were 284 women with different types of pregnancy complications. 129 of the women had given birth to LB. The control series consisted of 82 women with normal pregnancies matching in age and parity and without diabetic heredity. In the present follow-up study (series II) 82 of the women with LB and 52 of the controls could be re-tested. The interval between the tests was 5–7 years. The deliveries for both groups of women had taken place 3–32 years earlier (17.4 ± 6.2). Mean values are given with their standard deviations.

The mean ages of the women with LB in series I and II were 46.9 ± 6.9 and 52.6 ± 6.6 years respectively.

Table I. *Mean K-values of control mothers and mothers with LB in series I and II*

	n	Mean K Series I	Mean K Series II	p
Control mothers	54	2.22	1 65	<0 001
Mothers with LB	82	2.09	1.35	<0.001
Control mothers	54		1 65	<0.05
Mothers with				
LB+NW[a]	67		1 42	<0.02
LB+OW[b]	15		1 06	
LB+NW+DH[c]	25		1.36	n.s.[d]
LB+NW−DH	42		1 46	
LB+OW+DH	6		0.93	n.s.
LB+OW−DH	9		1 15	

[a] Normal weight.
[b] Overweight.
[c] Diabetic heredity
[d] Not significant.

The corresponding mean ages of the control group were 46.1±6.7 and 52.7±6.3 years respectively Methods were the same as given earlier (8). In the statistical calculations log K was used as the absolute values were not normally distributed (8) and the method of paired *t*-test was applied when comparing the results of series I with those of II. These calculations were performed only on corresponding values from the same women in series I and II.

RESULTS

The mean fasting blood glucose values in the control group increased between the tests from 81 9±6.5 to 86.8±6.2 mg/100 ml ($p<0\,001$) The women with LB showed a rise in mean fasting blood glucose value from 83 8±8 9 to 90 6±10.4 mg/100 ml during the 5–6 years. This difference was also highly significant ($p<0.001$) Figs. 1 and 2 show the frequency distributions of the fasting blood glucose values in the control group and in the group of mothers with LB at the time of the two tests. In both groups the blood glucose levels were higher at the second test.

In the control series there was a mean change in K values from 2.22±0.71 to 1 65±0.59 This decrease was highly significant (Table I). In the group of women with LB the mean K value decreased from 2.09±0 77 to 1.35±0.51 ($p<0\,001$) (Table I) The frequency distributions of K values in both groups are given in Figs. 1 and 2. The mean K-value of the women with LB was significantly lower ($p<0\,001$) than that of the control group at the repeat test (series II) but *not at the first test (series I)*

Among the women with babies of normal weight there were 4 (7%) who had developed K values below 1 0 In the group of women with LB, there were 20 (24%) with K values below 1 0. Of these, 3 (4%) already had K values below 1 0 at the first testing. Two women had developed overt diabetes. There was only *one* woman who increased her IVGT from a value below 1 0 to slightly above 1 0

The control group showed an insignificant slight increase in mean weight from 60.6±8.5 to 61.5±8.2 kg. On the other hand, the women with LB exhibited a significant ($p<0\,01$) increase from 70.5±11.2 to 72.2±12.9 kg. There was a significant difference ($p<0.001$) in mean height between the controls (162.1±5.3 cm) and the women with LB (166.5±5 7 cm)

Among the controls there was only one at the first and two at the second test who were overweight (30% above normal, (3)) Among the women with LB 18% were overweight. These overweight women were the same in series I and II Women with LB and overweight had a mean K value of 1 06 as compared to 1 42 in women of normal weight and LB ($p<0\,02$) The cor

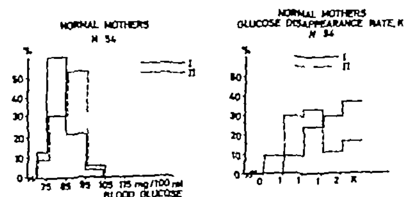


Fig. 1 The frequency distribution of fasting blood glucose values and K values in the control group.

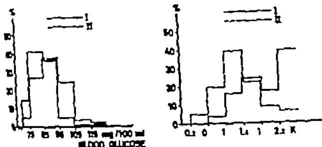


Fig. 2. The frequency distribution of fasting blood glucose values and K-values in women with large babies (LB).

responding mean K-values of series I were significantly higher (Table II). Out of the 20 women with LB and K-values below 1.0 7 (35%) were overweight. If controls of normal weight and mothers with LB also of normal weight were compared, the mean K-values of 1.65 and 1.42 respectively differed significantly ($p < 0.05$). These data together with some other comparisons are given in Table II. One of the controls had a first degree relative who had developed DM during the interval between the tests. There were 3 first degree relatives to the women with LB who had developed DM thereby increasing the frequency of diabetic relatives in this group from 24 to 28%.

DISCUSSION

The present results show that the IVGT after a time lapse of 5–7 years deteriorated both in women with normal obstetrical records and in those who had born LB. There was, however a difference between the two groups. In the control group only 7% developed K-values below 1.0 while 24% of the women with LB showed a reduced IVGT with K less than 1.0. Among the 129 women with LB tested the first time, 4 had developed DM since their last pregnancy. At this re-testing, 2 more women were found to have developed DM.

The present results are in accordance with those of two other studies (2, 17) which also showed deterioration of GT with time. Thus the five-year follow-up of the Ibstock population survey (17) demonstrated that 14% of those, who at the first testing had borderline OGTT had deteriorated and showed diabetic type curves. Manifest diabetes was found in 18 of 25 people previously found to have had a diabetic OGTT. The five year follow-up of the Birmingham diabetes survey of 1962 showed that out of the 13 subjects, who developed overt diabetes, 10 came from the group of 57 people who originally had diabetic type OGTT (2).

Published results (10, 12) are also in accordance with our findings which indicate that among women with LB the presence of obesity and/or diabetic heredity at the time of testing are important factors in determining abnormal glucose tolerance (Table I). Thus out of the 20 women with LB and K values below 1.0, 45% were overweight and 35% had a first degree-relative with DM. The study by Baird (1) further illustrated the interaction between obesity and diabetic heredity: in sibships of diabetic persons the percentage with one or more LB was more than twice that in sibships where the propositi were non-diabetics, and this high percentage was confined to those women who were obese. However obese women without diabetic sibling did not have the tendency to produce LB (1).

Other possible factors which could determine the outcome of the IVGTT must, however, be

Table II. *Change in IVGT during a 5–6 year period*

	K 1.0			
	1963–1965		1968–1970	
	No.	%	No.	%
Mothers with normal babies *N* 54	0	0	4	7
Mothers with large babies *N* 82	3	4	20	24

considered 9 out of 20 women with LB and all (4) women in the control group who had K values below 1 0 were not obese and did not have any first-degree relatives with DM.

The follow-up data discussed above are of little practical value in the clinical situation at the time of delivery It would be of prognostic and possibly preventive (9 15) value to be able to separate women who have born LB because of diabetic influences from those who have born LB because of a genetic predisposition to bear over weight infants (14) However IVGTT performed in mothers with LB shortly after delivery did not reveal a possible diabetic state (13) This finding does not exclude the possibility that some of these women might have had gestational diabetes since glucose tolerance usually reverts to normal immediately after delivery in women with known gestational diabetes (9 15)

In a recent study (13) we found that 21% of 129 overweight newborn infants had higher than normal K values and comparable to those found in infants of diabetic mothers (both gestational diabetics and insulin-treated mothers (7)) Follow-up studies of these mothers are necessary preliminaries to demonstrate the validity of the suggestion that the high K value of the infant will identify overweight infants who are the result of maternal diabetic influence during pregnancy

ACKNOWLEDGEMENTS

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MEASUREMENT OF FLOW RATE FOR THE STUDY OF UTERINE MOTILITY

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Abstract. A method for determining small rates and fluctuations of flow has been applied to the uterine secretions in non-pregnant women, the secretions being labelled by dextran, $4 \cdot 10^{-4}$ cal sec^{-1}. Base line secretory rates during consecutive phases of the menstrual cycle were determined but the main advantage of this technique is the unique possibility of studying fluctuations in the rate of flow as a measure of uterine motility without interference from foreign bodies in the uterine cavity. The responses of the non-pregnant human uterus in vivo to intravenously injected synthetic oxytocin and to the deposition of fluid in the posterior fornix are reported.

The motility of the non-pregnant human uterus in vivo has been described mainly from pressure measurements in the cavity of the uterine body and in the cervical canal, using sensors or open-ended, fluid filled catheters. The overall pressure peaks have been thought to represent uterine contractions, while the intracavitary pressure troughs have been referred to as the resting pressure or the tone of the uterus. This description has been accepted without major criticism, although theoretically a contraction of the myometrium can lead to increased volume and decreased pressure in both corpus and cervix simply by giving these preformed compartments a more spherical, cavitation shape.

In spite of the vast amount of work done in this field, the background to some clinical entities of great importance still remains to be more fully explained. One of these problems concerns the extent to which the myometrium of the non-pregnant uterus is involved in the phenomenon of reproduction, especially in cases of infertility with no anatomical basis, and another problem is the pathogenesis of dysmenorrhea. Information pertinent to these questions could possibly be gained by studying the fluctuations in the flow of secretions through the cervical canal during undisturbed conditions and following uterine stimulation. Because uterine secretions do not represent a true fluid but a rheological system, the effect of pressure changes upon the rate and direction of flow is unpredictable. This makes it tempting to suggest that the picture of uterine motility as reflected by recordings of the rate and direction of secretory flow through the cervix is of physiological significance and of at least equivalent interest to pressure recordings from the organ.

MATERIAL AND METHOD

Flow rate measurements according to a technique, described in detail elsewhere (5), were performed on 80 occasions in 18 women, 18 to 49 years old. None of the women, all of them volunteers, had symptoms of gynecological disease such as likely to influence ovarian or uterine function. In none of them were abnormalities detected on pelvic examination.

In 6 women, 3 of them nulliparous and 3 primiparous, all between 23 and 35 years old, the flow rate of uterine secretions was examined at 2 or 3 day intervals throughout the entire menstrual cycle. The phase of the cycle and the day of ovulation were verified from measurements of basal body temperature, colposcopic evaluation of the external os and fern tests of the cervical secretions (2, 3, 8).

In 6 of the volunteers, 24 to 47 years old (3 nulliparous, 2 primiparous and 1 multiparous) changes in the pattern of flow of uterine secretions were studied following intravenous injection of large doses of synthetic oxytocin. In each case measurements were performed twice during the cycle, once in the proliferative and once in the secretory phase; endometrial biopsy was performed for

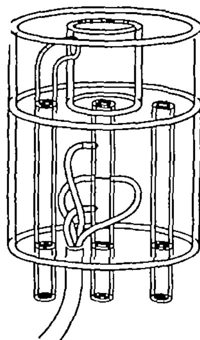


Fig 1 The receptor unit of the flowmeter based on detection of differential dispersion of thermal energy The thermistors are placed symmetrically around the heater in the central channel, which constitutes the outlet for the secretions.

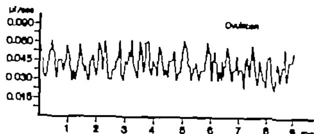


Fig 3 On the day of ovulation (same woman as in Fig. 2) the base line secretion rate is about 0.045 µl/sec and the fluctuations ± 0.015 µl/sec.

chamber was filled through the side arm with a 3.5% starch solution in water The starch solution was chosen because of its similar viscosity to uterine secretions. When no air bubbles remained in the system, the side arm and the central channel of the transducer were closed and the phase splitter adjusted in order to obtain a symmetrical output signal for the bridge. Current was passed through the heating element and the output signal rendered isoelectric by adjusting th. potentiometers in the bridge circuit and in the varistor systems (5). The transducer channel was opened, the subject was asked to assume a comfortable supine position, and the recording was begun.

verification of the phase Twenty to forty mU/kg of oxytocin (Syntocinon, Sandoz) w re given over a period of 30 sec, the volume of the injection always being 2.5 ml.

In 6 women, 33 to 47 years old (2 nulliparous and 4 multiparous) the change in flow rate was studied following injectio of isotonic sodium chloride solution at body temperature into the posterior fornix in order t determine whether this influences uterine ctivity The solution was injected via a polyethylene tube (less than 2 mm in di ameter) ending in the posterio vaginal fornix abov the plane of the transducer Three recordings were made in the proliferatıv phase and three in the secretory phase endometrial biopsy being perf rmed f confirmation.

Prior to the measurements the subject was placed in lithotomy positio and the cervix xposed. The chamber fo collection of the secretions was attached to the cerv by suction from water jet pump. Fig. 1 The transducer



Fig 2 Recording of the flow rate (about 0.035–0.040 µl/sec) and the superimposed fluctuations (about ± 0.007 µl/sec) fo a 3-year-old nulliparous woma during the proliferative phase

RESULTS

The base line secretion rate upon which the fluctuation of flow rate was superimposed varied considerably from case to case In the individual case the secretory rate changed gradually during the course of the cycle, maximal values being reached in mid-cycle One series of recordings shows that the base line secretion rate during the proliferative phase is about 0 035–0 040 µl sec $^{-1}$ (Fig 2) and on the day of ovulation $0\,045 \pm 0.015$ µl sec $^{-1}$ (Fig. 3) during the secretory phase (Figs. 4 and 5) the secretion rate is 0 035 µl sec $^{-1}$ 4 days before menstruation and about 0 020 µl sec $^{-1}$ 2 days before menstruation.

The pattern of flow fluctuations displayed a tendency to change during the course of the menstrual cycle In the proliferative phase the fluctuations were frequent but small in Fig. 2 the frequency ranges between 5 and 10 per min and the amplitude amounts to about ± 0.007 µl sec $^{-1}$ On the day of ovulation, increased amplitudes were observed in a few cases (an example is gi en in Fig. 3) During the late secretory phase both the interval between peaks and their magnitude increased In Figs. 4 and 5 the frequency of peaks, reflecting a change in flow

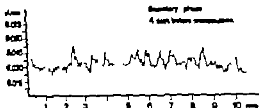


Fig. 4. Recording during the secretory phase, 4 days before the onset of menstruation (same woman as in Figs. 2 and 3). The base line secretion rate is 0.035 μl/sec and fluctuations ±0.010 μl/sec.

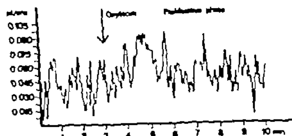


Fig. 6. Recording from 39-year-old multiparous woman during the proliferative phase. Synthetic oxytocin, 20 mU/kg, was administered intravenously for 30 sec at the time indicated by the arrow. The injection is followed by an accelerated flow of secretions, with return to the original flow pattern 3 min after the injection.

of at least 0.005 μl sec ranged between 3 and 5 per min. In the days prior to the onset of menstruation maximum fluctuations reached values of ±0.035 μl sec. Reversed flow (i.e. inflow to the uterus) was also recorded at this time.

The effect of oxytocin (large doses of synthetic oxytocin injected i.) was detectable on only three of the twelve occasions studied, one being in the proliferatis phase (Fig. 6) and two in the secretory phase, close to the start of menstruation (Figs. 7 and 8). The change in uterine motility appeared less than 1.5 min after the injection. In the other nine recordings there was either no distinct response or the change in motility occurred after such long interval that it could not be attributed to the action of oxytocin upon the myometrium.

The most pronounced finding in the three cases in which response to oxytocin occurred, was an increase in the rate of flow. I two of these cases the values approached 0.10 μl sec representing an increase of several times the base line secretion rate (Figs. 7 and 8). The response was transient in two cases, lasting only about 2 or 3 min (Figs. 6 and 7), followed by return to the pattern of uterine activity at the pre-injection secretory rate. In the *third* instance the response was more sustained, flow fluctuations occurring around an elevated base line for more than 5 min (Fig. 8).

Observations of flow rate as measure of uterine response to the injection of saline in the posterior vaginal fornix revealed that the response was most marked in the proliferative phase and that an immediate reduction of the outward flow followed by an increase to or abov the initial base line levels, can occur (Fig. 9). Apparently reversal of flow can also take place: Fig. 10 illustrates a rapid retardation of outward flow followed by a transient flow into the uterus. Fig. 11 however shows that the injection can also be followed simply by an increased outward flow.

DISCUSSION

The main advantage of measuring uterine motility by studying the variations in the rate of flow

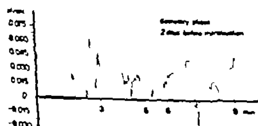


Fig. 5. Recording during the secretory phase, 2 days before menstruation. The base line secretion rate is 0.022 μl/sec and the superimposed fluctuations vary to marked degree, maximal values being 0.035 μl/sec. There are also short periods of inflow to the uterus.

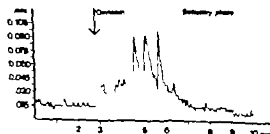


Fig. 7. Recording of flow in 24-year old nulliparous woman during the secretory phase. Synthetic oxytocin, 20 mU/kg, was given intravenously at the arrow. There is marked response, involving an increased rate of flow and large fluctuations. The pre-injection pattern returns within 4 min.

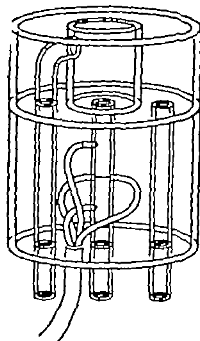


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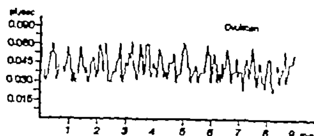


Fig 3 On the day of ovulation (same woman as in Fig. 2) the base line secretion rate is about 0.045 µl sec and the fluctuations ±0.015 µl/sec.

chamber was filled through the side arm with a 3.5% starch solution in water The starch solution was chosen because of its similar viscosity to uterine secretions. When no air bubbles remained in the system, the side arm and the central channel of the transducer ere closed and the phase splitter adjusted in order to obtain a symmetrical output signal for the bridge Current was passed through the heating element and the output signal rendered isoelectric by adjusting th. potentiometers in the bridge circuit and in the arístor systems (?) The transducer channel was opened, the subject was asked to assume a comfortable supine position, and th. recording was begun.

enification of the phase T enty to forty mU kg of oxytocin (Syntocinon, Sandoz) were gi en over period of 30 sec, the volume of the injection alw ys being ..5 ml.

In 6 w men, 33 to 47 years old (nulliparous and 4 multiparous) the change in flow rate was studied following injection of isotonic sodium chloride solution at body temperature into the posterior fornix in order t determine whether this influences uterine activity The solution was injected ia a polyethylene tube (less than mm in diameter) ending in the posterior vaginal f rnix above the plane of the transducer Three recordings were made in the proliferati phase and three in the secretory phase, endometrial biops being performed for confirmation.

Prior to the measurements the subject was placed in lithotomy position and the cerv x exposed. The chamber for collection of the secretions was attached to the cervi by suction from water jet pump, Fig. 1 The transducer



Fig Recording of the flow rate (about 0.035–0.040 µl sec) and the superimposed fluctuations (about ±0.007 µl/sec) for a ..3-year-old nullipar-us woman during the proliferati phase

RESULTS

The base line secretion rate upon which the fluctuation of flow rate was superimposed varied considerably from case to case. In the individual case the secretory rate changed gradually during the course of the cycle maximal values being reached in mid-cycle One series of recordings shows that the base line secretion rate during the proliferative phase is about 0 035–0 040 µl sec ¹ (Fig ..) and on the day of ovulation 0 045 ± 0.015 µl sec ¹ (Fig 3) during the secretory phase (Figs. 4 and 5) the secretion rate is 0 035 µl sec ¹ 4 days before menstruation and about 0 020 µl sec ¹ 2 days before menstruation

The pattern of flow fluctuations displayed a tendency to change during the course of the menstrual cycle In the proliferati e phase the fluctuations were frequent but small in Fig the frequency ranges between 5 and 10 per min and the amplitude amounts to about ~0 007 µl sec On the day of ovulation increased amplitudes were observed in a few cases (an example is given in Fig. 3). During the late secretory phase both the interval between peaks and their magnitude increased In Figs 4 and 5 the frequency of peaks, reflecting a change in flow

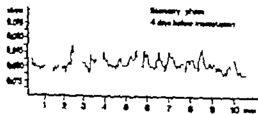


Fig 4 Recording during the secretory phase, 4 days before the onset of menstruation (same woman as in Figs. 2 and 3) The base line secretion rate is 0.035 µl/sec and fluctuations 0.010 µl/sec.

of at least 0.005 µl sec ranged between 3 and 5 per min In the days prior to the onset of menstruation maximum fluctuations reached values of +0.035 l sec Reversed flow (i.e. inflow to the uterus) was also recorded at this time.

The effect of oxytocin (large doses of synthetic oxytocin injected i.) was detectable on only three of the twelve occasions studied, one being in the proliferative phase (Fig. 6) and two in the secretory phase close to the start of menstruation (Figs. 7 and 8) The change in uterine motility appeared less than 1.5 min after the injection. In the other nine recordings there was either no distinct response or the change in motility occurred after such a long interval that it could not be attributed to the action of oxytocin upon the myometrium

The most pronounced finding in the three cases in which a response to oxytocin occurred, was an increase in the rate of flow I two of these cases the values approached 0 10 µl sec representing an increase of several times the base line secretion rate (Figs. 7 and 8). The response was transient in two cases, lasting only about 2 or 3 min (Figs. 6 and 7), followed by a return to the pattern of

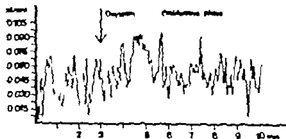


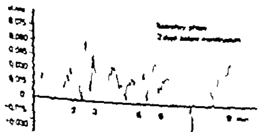
Fig 6. Recording from 39-year-old multiparous woman during the proliferative phase. Synthetic oxytocin, 20 mU/kg, was administered intravenously for 30 sec at the time indicated by the arrow The injection is followed by an accelerated flow of secretions, with a return to the original flow pattern 3 min after the injection.

uterine activity at the pre-injection secretory rate. In the third instance the response was more sustained, flow fluctuations occurring around an elevated base line for more than 5 min (Fig. 8)

Observations of flow rate as measure of uterine response to the injection of saline in the posterior vaginal fornix revealed that the response was most marked in the proliferative phase and that an immediate reduction of the outward flow followed by an increase t or above the initial base line levels, can occur (Fig 9). Apparently reversal of flow can also take place Fig 10 illustrates a rapid retardation of outward flow followed by a transient flow into the terus, Fig. 11 however shows that the injection can also be followed simply by an increased outward flow

DISCUSSION

The main advantage of measuring uterine motility by studying the variations in the rate of flow



F 5 Recording during the secretory phase, days before menstruation The base line secretion rate is 0.022 µl sec and the superimposed fluctuations vary to marked degree, maximal values being 0.01 l sec. There are ... short periods of inflow to the uterus.

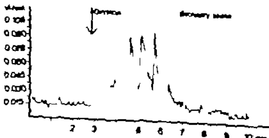


Fig 7 Recording of flow in 24-year old multiparous woman during the secretory phase Synthetic oxytocin, 20 mU kg, was given intravenously at the arrow There is marked step up, involving an increased rate of flow and large fluctuations. The pre-injection pattern returns after 4 min



Fig 8 Recording (late secretory phase) on the same 24-year-old multiparous woman as in Fig. 7 Synthetic oxytocin, 40 mU/kg, was given at the arrow The injection is f llowed by a sustained response with an increased mean secretory rate and a marked increase in the amplitude of fluctuations.

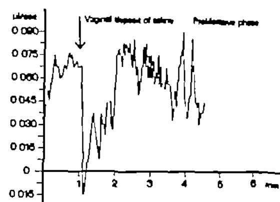


Fig 10 Following intravaginal injection of saline at body temperature (at the arrow) there is a short period of inflow of the cervix secretions. Proliferative phase.

of uterine secretions is that no foreign bodies or substances are introduced into the uterine cavity The receptor of the flowmeter is gently attached to the vaginal cervix by means of reduced pressure. It is known from clinical experience that this part of the cervix is highly insensitive to trauma. With this method, therefore, the motility of the uterus can be studied under relatively undisturbed conditions.

Little specific information can be obtained from the literature about the secretory rates of cervical and endometrial glands (8 10 11) Collecting the secretions by aspiration once or twice a day gave daily amounts corresponding to flow rates of 0 002–0.007 μl sec $^{-1}$ during midcycle Using gravimetric methods to estimate the total amount of material secreted per day around ovulation Odeblad (7) derived values 10 times higher or equivalent to 0 05 μl sec^{-1} This is in accordance with the values found in the clinical application of this method. Virtually nothing is known about spontaneous variations in the secretory rates (6) or the possibility of inducing changes by means of pharmacologically active substances. Judging from other glandular structures it seems unlikely however that changes in secretory rate would be sufficiently large to interfere with the evaluation of changes in flow rate due to uterine motility This study shows moreover that the baseline secretory rate changed little during the period of measurement. Even when the rate of secretion changed in some experiments there was no difficulty in assessing the superimposed fluctuations caused by uterine myometrial activity

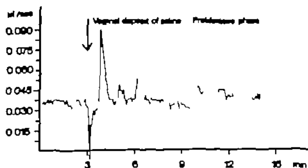


Fig 9 Recording of flow through the cervix in 36-year-old multiparous woman Three ml of saline t body temperature were injected in the posterior vaginal f rnix at the arrow The injection is followed by ah rt period of inflow and a short period of increased outflow of the secretions. The pre-injection base line secretory rate then returns, the fluctuations also being about the same as before the injection Pr liferative phase

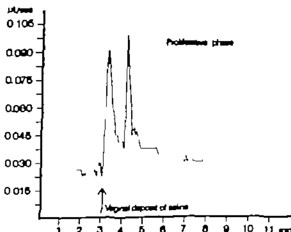


Fig 11 Recording on 37 year-old multiparous woma Following intravaginal injection of 3 ml saline at body temperature the outflow rate is significantly levated for almost 3 min. Even when the pattern has been readjusted, the base line secretion rate is higher than the pre-injection level. *Proliferative phase*

The possibility of determining variations in uterine volume without changing the pressure artificially means that information can be obtained which is not available with conventional techniques for studying uterine motility. Data on relative pressures in the two compartments of the uterus and in the vagina do not suffice to predict the effect of motility upon the rate and direction of flow of the uterine secretions since no correlation has been established between myometrial activity and volume changes in the corpus and cervix. Although it seems likely that most uterine contractions are accompanied by a decrease in volume, situations can exist where the total intracavitary volume is unchanged or increased. The combined effect of localized contractions and relaxations of different parts of the myometrium could be responsible for this. The volume of a hollow organ can also increase if a generalized contraction tends to give the organ a more spherical shape. Furthermore, the complex character of the uterine secretions is responsible for incalculable movements of the secretions in the cervical canal.

A diagram of fluctuations in the rate of flow through the cervical canal obviously does not correspond to the pattern of non pregnant uterine motility reproduced by e.g. pressure measurements with the balloon method. The flow diagrams serve to summarize the modulating effect of myometrial activity with subsequent volume and pressure changes, on the rate of flow of uterine secretions.

Although the individual variations are considerable, typical examples are given to show the changes in secretory rate during consecutive phases of the menstrual cycle. The superimposed fluctuations due to pressure and volume changes in the corpus and cervix of the uterus, and associated with uterine motility are observed to assume patterns that may be characteristic for the various phases of the menstrual cycle. The susceptibility of the uterus to intravenously administered synthetic oxytocin (1, 4) is demonstrated also with the present method. Furthermore it is tempting to suggest, that the diminished flow of secretions—or in some instances the reversal of flow—which is a result of fluid deposition in the posterior vaginal fornix, might be relevant in connection with problems of sperm transport and fertility.

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ångest, spanning, rastloshet och somnloshet ar symtom på att den psykiska balansen ar stord

DIAZEPAM DUMEX

Stesolid

för psykisk balans

tabletter 2,5 och 10 mg   mixtur 0,4 mg/ml   ampuller 5 mg/ml

	Angest, oro och angslan (foretradesvis vid neurotiska tillstånd)	Sömnrubbningar	Muskel spänningar
1— 3 år	½—1 mg x 3	1—2 mg till natten	Vid CP och atetos individ dosering
4—14 år	1—2 mg x 3	2—4 mg till natten	Vid CP och atetos individ dosering
15—60 år	Ambulant initialt 2 mg x 3	5—15 mg till natten	2—5 mg x 3—4
> 60 år samt svaga patienter	2 mg x 2 (ev for siktig dosökning under observ)	2 mg till natten	2 mg x 2

Doserna kan efter initialbehandling med låg dosering justeras efter individuellt behov och tolerans. Dygnsdosen 20 mg bor overskridas endast på str ng ind kation

) Vid samtidigt inslag av somnsvårigheter rekommenderas att halften av dagsdosen ge på kvällen och halften 1 eller 2 enkeldoser nder dagen

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for övriga upplysningar se FASS

DUMEX

PROLAPSE OF THE UMBILICAL CORD

Johannes E. Bock and J. Wiese

Department of Obstetrics B and Department I Gynaecology M (Heads: Mogens Osler and Vagn Sele. Former head: E. Brandstrup), Rigshospitalet University of Copenhagen, Denmark

Abstract. Two series of cases of umbilical cord prolapse are submitted. One comprises 97 cases and the other 64. The total corrected mortality rate was 13.0% and 34.5% respectively, the mature corrected mortality rate 1.5% and 39%. The total mortality for breech presentations with prolapsed cord was 19.2% and 23.8%, respectively. In cephalic presentations the mortality rate was 10.3% and 45% respectively. This difference in mortality is explained by a more active attitude in the department having the lower mortality, where the diagnosis was more often made by vaginal examination, the delivery was more rapid, and the use of Caesarean section more common.

During the past few decades there has been a marked decrease in perinatal mortality. In Denmark the rate was 1.8% in 1968. In an effort to reduce it even further the individual contributory factors must be analysed.

The mortality in umbilical cord prolapse is high, but in the course of time it has shown a falling trend. Du Toit (7), in 1956, found a perinatal mortality rate of 32.0%. Cushner (3) in 1961 41.7%. Later Widholm & Nieminen (8) found a perinatal mortality rate of 10.7%. Brant & Lewis (1) 11.4% and Pathak (6) 19.3%.

According to Cushner's (3) review of the literature from 1932 to 1959 the incidence of umbilical cord prolapse is 0.43%, i.e. one case in 233 deliveries. Thus, prolapse of the umbilical cord is an important factor in the total perinatal mortality.

Below we are submitting the cases of umbilical cord prolapse seen in two departments over a 10 year period and an analysis of the influence of therapeutic factors upon the mortality rate.

MATERIAL AND RESULTS

During the decade 1.4.1958 to 31.3.1968 a total of 19 152 infants over 1 000 g were born in Obstetrical Dept. A and 21 540 in Obstetrical Dept. B of Rigshospitalet, University of Copenhagen. From Table I it may be seen that in Dept. A the incidence of umbilical cord prolapse was 0.5% and in Dept. B 0.3%. Furthermore, it is apparent that the incidence is higher among premature than among mature infants. Infants weighing less than 1 000 g were excluded, as the high overall mortality in this group does not permit analyses of the role of umbilical cord prolapse. Within the series there is slight preponderance of multiparas, viz. 56.7% in Dept. A and 54.7% in Dept. B.

Table II gives the incidence of umbilical cord prolapse in various presentations. It shows that this complication is more common in breech presentations and transverse lies than in cephalic presentations. is also more common in multiple pregnancies, occurring in 12 cases (3.3%) in Dept. A and in 8 (2.3%) in Dept. B. Table III partly

Table I. *Incidence of cord prolapses 1958–1968*

Weight (g)	Department A		Department B	
1 000–2 499	29	1.03	25	0.88
2 500 or over	68	0.42	39	0.21
Total	97	0.51	64	0.30

Table II. *Incidence of cord prolapse in relation to presentation*

Presentation	Department A No of cases	Department A Risk of prolapse ( )	Department B No of cases	Department B Risk of prolapse (%)
Occipital	35	0.21	35	0.18
Sincipital brow and face	4	1.59	0	
Breech	52	3.63	21	1.59
Oblique and transverse	6	3.59	8	7.34

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for övriga upplysningar se *FASS*

DUMEX

Acta Obstet Gynec Scand 51 303–308, 1972

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During the decade 1.4.1958 to 31.3.1968 a total of 19 132 infants over 1 000 g were born in Obstetrical Dept. A and 21 543 in Obstetrical Dept. B of Rigshospitalet, University of Copenhagen. From Table I it may be seen that in Dept. A the incidence of umbilical cord prolapse was 0.5% and in Dept. B 0.3%. Furthermore, it is apparent that the incidence is higher among premature than among mature infants. Infants weighing less than 1 000 g were excluded, as the high overall mortality in this group does not permit analysis of the role of umbilical cord prolapse. Within the series there is a slight preponderance of multiparae, viz. 56.7% in Dept. A and 54.7% in Dept. B.

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Presentation	Department A: No. of cases	Department A: Risk of prolapse ( )	Department B: No. of cases	Department B: Risk of prolapse (%)
Occipital	35	0.21	33	0.16
Sincipital brow and face	4	1.39	0	
Breech	52	3.63	21	1.59
Oblique and transverse	6	3.59	8	7.34

Table III *Fetal mortality in cord prolapse in relation to birth weight*

Weights (g)	Department A			Department B		
	No. of cases	Deaths ( )	Mortality ( )	No. of cases	Deaths ( )	Mortality (%)
1 000–1 499	12	11	91 7	8	6	75.0
1 500–1 999	6	3	50.0	8	6	75 0
2 000–2 499	11	0	0.0	9	3	33.3
2 500 or over	68	3	4 4 (1.5)	39	11	28.2 (1 4)
Total	97	17	17.5 (4 4)	64	26	40.6 (5 6)

Figures in parentheses represent the overall perinatal mortality

Table IV *Corrected fetal mortality in cord prolapse*

Weights (g)	Department A			Department B		
	No. of cases	Deaths (n)	Mortality (%)	No. of cases	Deaths ( )	Mortality ( )
1 000–2 499	26	11	4_.3	23	13	56.5
2 500 or over	66	1	1.5	35	7	20 0
Total	92	1	13.0	58	20	34.5

sets out the total mortality rate in umbilical cord prolapse and partly the mortality rate in the various birth weight groups. This clearly shows a difference in mortality between the two departments, the total perinatal mortality rate in umbilical cord prolapse being 17.5% in Dept. A and 40.6% in Dept. B, and for the eight group ≥ 500 g it was 4 4% and 28_ respectively After exclusion of infants who had died before admission and infants who had fatal malformations, the corrected mortality rate was as shown in Table IV From this table it is apparent that the corrected mortality for infants ≥ 2 500 g was only 1.5 in Dept. A. The total perinatal mortality rate in this weight group was also 1.5 in Dept. A (cf. Table III).

To elucidate these differences in the mortality and the difference in the number of cases with prolapsed cord in the weight group ≥ 500 g the fetal mortality was analysed fo cephalic presentations and fo breech presentations separately in the arious weight groups (Tables V and VI).

From these tables it is apparent that the difference in the total mortality rate applies only to the cephalic presentations (10.3% in Dept. A and 54.3% in Dept. B), hereas no definite difference is demonstrable for the breech presentations (9_% and _3.8%).

There were considerably large number of breech presentations with prolapsed cord in Dept. A than in Dept. B, but there was no major difference in the number of cephalic presentations with cord prolaps.

Table VII lists the corrected fetal mortality in cephalic presentations. In Dept. A it was 5 4% and in Dept. B 48.4 It is remarkable that no infant eighing more than 500 g died in Dept. A.

Table VIII shows that prolapse of the umbilical cord was diagnosed more often by routine vaginal examination in Dept. A than in Dept B and that as a whole

Table V *Fetal mortality in cord prolapse cephalic presentation*

Weights (g)	Department A			Department B		
	No. of cases	Deaths ( )	Mortality ( )	No. of cases	Deaths ( )	Mortality ( )
1 000–1 499		1	50.0	4	3	75
1 500–1 999	3	1	33.3	3	3	100
2 000–2 499	5	0	0	6	3	50
500 or over	29		6.9	—	10	45.5
Total	39	4	10.3	35	19	54 3

Table VI. *Fetal mortality in cord prolapse: breech presentation*

Weight (g)	Department A			Department B		
	No. of cases	Deaths (n)	Mortality (%)	No. of cases	Deaths (n)	Mortality (%)
1000-1499	9	9	100	4	3	75
1500-1999	1	0	0	2	2	100
2000-2499	6	0	0	3	0	0
2500 or over	36	1	2.9	12	0	0
Total	52	10	19.2	21	5	23.8

Table VII. *Corrected fetal mortality in cord prolapse: cephalic presentation*

Weight (g)	Department A			Department B		
	No. of cases	Deaths (n)	Mortality (%)	No. of cases	Deaths (%)	Mortality (%)
1000-2499	10	2	20.0	12	8	66.7
2500 or over	27	0	0	19	7	36.8
Total	37	2	5.4	31	15	48.4

the mortality was lowest when the diagnosis was made by this means.

The relationship of the time factor to the mortality rate may be seen from Table IX. Comparison of the perinatal mortality rate among infants delivered within half an hour and among those delivered more than 1 hour after the diagnosis had been made shows a doubling of the mortality rate in both departments. At the same time, it will be noted that the majority of the infants in Dept. A were delivered within half an hour, whereas in Dept. B the majority were delivered more than 1 hour after the diagnosis.

Tables X and XI set out the fetal mortality in cephalic and in breech presentations in relation to the degree of cervical dilatation and the use of Caesarean section.

Table X reveals that for each degree of cervical dilatation there was a considerably higher fetal mortality rate for cephalic presentations in Dept. B than in Dept. A. Correspondingly the use of Caesarean section in the individual groups was more common in Dept. A than in Dept. B. It may be mentioned that all the 5 surviving infants in Dept. B belonging to the group where the cervix was 5-8 cm dilated were delivered by Caesarean section.

As far as the breech presentations are concerned, Table XI shows that there was no essential difference in mortality between the two departments. It should be mentioned that all the dead infants weighed less than 1 500 g, except one (2 800 g) delivered in Dept. A.

DISCUSSION

The incidence of umbilical cord prolapse in the two departments (0.5% and 0.3%) accords with the findings of Cushner (3) (0.48%) and of Widholm & Nieminen (8) (0.41%).

The total mortality rate in Dept. B was 40.6%, corrected 34.5%, i.e. similar to du Toit's (7) of 32.0% and Cushner's (3) of 41.7%. But there are more recent series with a considerably lower mortality. For instance, Widholm & Nieminen

Table VIII. *Fetal mortality related to diagnostic method*

Method	Department A			Department B		
	No. of cases	Deaths (n)	Mortality (%)	No. of cases	Deaths (n)	Mortality (%)
Vaginal	62 (63.9 %)	9	14.5	29 (45.3 %)	9	31.0
Fetal heart rate	5 (5.2 %)	0	0.0	15 (23.4 %)	8	53.3
Visual	30 (30.9 %)	8	26.7	19 (29.4 %)	9	47.4
Other	0			1	0	

Table IX. *Fetal mortality related to time between diagnosis and birth*

Time (min)	Department A			Department B		
	No. of cases	Deaths ( )	M rtality (%)	No of cases	Deaths ( )	Mortality (%)
0–30	57	8	14.0	21	5	23.8
30–60	17	2	11.8	5	3	60 0
Over 60	23	7	30.4	38	18	47 4

Table X. *Fetal mortality related to cervical dilatation and Caesarean sections in cephalic presentations*

Cervical dilatation (cm)	Department A				Department B			
	No. of cases	Deaths	Mortality (%)	Caesarean section (%)	No. of cases	Deaths	Mortality (.)	Caesarean section (%)
0–4	11	0	0	72.7	16	9	56.2	37.5
					(14)	(7)	(50.0)	
5–8	8	3	37.5	75	10	5	50 0	50
	(6)	(1)	(16.7)		(8)	(3)	(37.5)	
9–10	19	1	5.3	10.5	9	5	55 6	0
Unknown	1	0	0					

Figures in parentheses represent the corrected number and mortality

(8) reported a corrected perinatal mortality rate of 10.4% Brant & Lewis (1) 11 4% and Clark et al. (2) 16.8% These figures are comparable to the mortality in Dept. A (total 17.5% and corrected 13 0%)

When considering only the perinatal mortality rate for mature infants (≥ 2 500 g) it was 28.2% in Dept. B and 4 4% in Dept. A. Clark et al. (2) found a mature mortality rate of 4.5% Widholm & Nieminen (8) 9 4%

The corrected mortality rate for the same weight group was 20 0% in Dept. B and 1.5% in Dept. A. This latter mortality does not differ from the total mortality in Dept. A (1 5%) for the over 2 500 g group.

This leads us to consider whether we ca detect factors which determine this difference i mortality between Depts. A and B

During the study period there were 52 breec presentations with cord prolapse, or 3 6% of al deliveries, in Dept. A, whereas only 21 or 1 6% had occurred in Dept. B The total mortality fo breech presentations with prolapsed cord wa approximately the same in both departments, viz. 19.2% in Dept. A and 23 8% in Dept. B This mortality is close to that reported by Clark et al. of 21% whereas Widholm & Nieminen (8) lost only 12% As already mentioned there was only one death among mature infants presenting by the breech. This baby was delivered by Caesarean

Table XI *Fetal mortality related to cervical dilatation and Caesarean sections in breech presentations*

Cervical dilatation (cm)	Department A				Department B			
	No. of cases	Deaths ( )	Mortality (.)	Caesarean section (%)	N of cases	Deaths ( )	Mortality (")	Caesarean section ( )
0–4	10	4	40	70	6	2	33.3	66 7
	(9)	(3)	(33.3)					
5–8	10	0	0	90	4	2	50	0
9–10	32	6	18.8	0	11	1	9 1	9 1
	(30)	(4)	(13.3)					

Figures in parentheses represent the corrected number and mortality

section, but not until 1 hour 15 minutes after the diagnosis had been made.

The number of cephalic presentations with umbilical cord prolapse was approximately the same in both departments, 39 in Dept. A and 35 in Dept. B and it is in these cases that there is an obvious difference in mortality.

Thus, the total mortality rate in Dept. A was 10.3% corrected 5.4% i.e. comparable with the results of Widholm & Nieminen (8) and Clark et al. (2) of 13.5% and 7.5% respectively whereas the total mortality rate in Dept. B was 54.3% corrected 48.4%. It must be pointed out that the corrected mortality for mature infants in Dept. A was 0 and in Dept. B 36.8%.

Now what is the explanation of this difference in mortality?

Prolapse of the umbilical cord was diagnosed more often by vaginal examination in Dept. A than in Dept. B (cf. Table VIII). When the diagnosis is made by this means the mortality is lowest, as has been reported by others including Clark et al. (2) and Cushner (3).

The majority of the infants in Dept. A were delivered within half an hour after the diagnosis had been made whereas in Dept. B the majority were delivered more than 1 hour later. This doubled the mortality. The role of the time factor has also been elucidated by Widholm & Nieminen (8) who found a mortality of 13.1% at less than 30 min and of 20.5% at more than 30 min.

In Dept. A the use of Caesarean section in cephalic presentations was more common than in Dept. B at all degrees of cervical dilatation, and the mortality was correspondingly lower in Dept. A. This is in conformity with the findings of many others (Brant & Lewis (1), Clark et al. (2), Daly and Gibbs (4), Pathak (6), Cushner (3)).

All these findings indicate a more active attitude to the treatment of cord prolapse in Dept. A than in Dept. B. That this makes the mortality lower in Dept. A corresponds to the finding of Nirwander et al. (5) who reported a mortality rate of 11.8% with active treatment as compared with 72.4% with a passive attitude to prolapse of the umbilical cord.

This is further confirmed by analysing the deaths of the 7 mature infants in Dept. B (Table VII). It is apparent that in 6 out of the 7 cases more active obstetrical treatment might have prevented the fetal death. Thus, in two cases with a cervical dilatation of 5–6 cm vacuum extraction was applied for $1^1/_2$ and $2^1/_2$ hours respectively (fetal weights 3 600 and 4 500 g). In two cases Caesarean section was delayed, for 1 hour 15 min and for 1 hour 50 min. In one of the cases replacement of the cord was also tried. In one case vaginal examination was not performed until 6 hours after the membranes ruptured, and an attempt at replacing the cord was made. One patient started vomiting while Caesarean section was being prepared, and the operation was therefore cancelled. It should be mentioned that during the last 5-year period, while the attitude in Dept. B has been more active, only one mature infant has died.

On the basis of what has been stated above it may be concluded that an active attitude to the management of umbilical cord prolapse results in the lowest mortality. In other words, the following factors are of importance:

Frequent vaginal examinations, and always immediately after the membranes have ruptured.

Quick delivery (i.e. Caesarean section) in all cases where the cervix is incompletely dilated and where vaginal delivery cannot be effected quickly and easily—provided that the fetus is alive, does not have fatal malformations, and is not immature. Until the Caesarean section can be carried out, the presenting fetal part should be held up as recommended by Brant & Lewis (1) to avoid compression of the cord.

By acting on these lines there ought to be a possibility of reducing the mortality rate in umbilical cord prolapse to approximately the average perinatal mortality.

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ACRIDINE ORANGE IN GYNECOLOGIC CANCER

I. *The Proton Magnetic Resonance Spectrum of Acridine Orange Zinc Chloride*

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act With the ultimate purpose of evaluating the ...cy and specificity of the fluorescence of acridine ...e stained cells or tissues as ... sign of malignancy ...compound and its binding to stain receptors have ...studied with biophysical methods. The present paper ...bes the identification of the lines in the proton ...etic resonance spectrum of acridine orange zinc ...oride. The ring proton lines are weak but the methyl ... strong and easy to record with ... high ratio of ...signal-to-background noise. The methyl line is com... of two superimposed parts, one narrow and one ..., with the same chemical shift. The reason why the ...l line is divided into components is, however, still ...own.

...dine orange was described as an aid to the ...r colposcopic diagnosis of cancer by Hinsel...n (1954). Its use as ... stain for ...aginal smears ...simplify screening was introduced by von ...alanffy and coworkers (1958). The brilliant ...nge-red fluorescence in ultraviolet light was ...ght to be specific for malignant squamous ...helial cells, but it was later found that fibrin ... necrotic tissue gave the same fluorescence ...physical studies on the binding of acridine ...nge to different stain receptors may help to ...lain ...arious fluorescent phenomena and thus ...n ... basis for clinical applications.

...everal stains used in gynecologic exfoliativ... ...ology have been studied with nuclear magnetic ...onance (NMR) techniques (Höglund & Odeblad, ...'2). Some of these stains giv... rise to narrow ...ctral lines facilitating detailed studies of molec... ...r interactions in stain absorption processes. ...e stain showing narrow NMR lines is acridine ...ange (Fig. 1). I... this first report on ... series of experiments utilizing biophysical techniques ele...mentary information on some NMR properties of acridine orange will be presented.

METHODS

NMR spectra of solutions of acridine orange in D_2O were studied ...ith ... Varian T-60 spectrometer for protons at 60 MHz. The instrument was adjusted to very high resolution (0.018 Hz) or 0.003 ppm line width. The recordings were performed under nonsaturation conditions. Because of the fairly broad lines, the standard error for determination of shifts was ± 0.04 ppm. The standard error of the height of the methyl line in acridine orange was $\pm 4\%$. The voltage ratio between signal and background noise ...as determined to about 30 for the methyl line of ... 2.5% acridine orange solution.

The spectrometer was equipped with an electronic integrator circuit. Thus, integrated spectra could be superimposed on NMR absorption spectra. The spectrometer also had facilities for selective irradiation of separate proton lines ...ith high intensity RF radiation for spin decoupling studies.

MATERIALS

The solutions of acridine orange in D_2O ...ere 3.3% or ...3%. The solubility has been estimated to 5% in literature, but somewhat smaller concentrations were used in order to avoid excessive molecular association effects. The heavy ...ater contained about 0.5% of H_2O as impurity. Additional H_2O from air moisture may also contaminate the samples during handling.

The acridine orange used ...as supplied by Merck Chemical Co. CI No. 46005 SNo 902. The formula is $C_{17}H_{20}Cl_3N_3Zn$, molecular ...eight 438.10. The quality is "for microscopy applications". It is present in the form of zinc chloride double salt.

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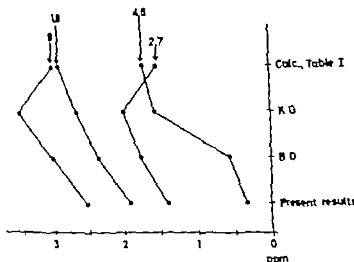


Fig. 3. A comparison between calculated and experimental shifts of ring protons in acridine orange. KG = Kokko & Goldstein (1963) BD = Blears & Danyluk (1967)

virtually disappeared. If samples were stored at +4 C, +30 C, +63 C and +100 C for four hours, the line intensities were 1.3 1.2, 0.2 and 0 respectively the H 9 intensity being unity. If a sample was stored at +65 C and investigated at various points in time the line intensity showed decay c f. Table II. This behaviour suggests that there may be an exchange of the 4 and 5 protons of the acridine orange molecule with the surrounding heavy water within a measurable time (half-time about 1.0 hours at +65 C)

3. Structure of the methyl line

On an expanded scale the methyl line showed a deviation from the expected Lorentz shape (Fig. 4), which indicates that it is composed of two superimposed components. The chemical shift between these parts was determined and was found to be 0.002 ± 0.007 ppm. Thus they can be regarded as having the same shift. The integrated intensity ratio of the narrow to the broad component was about 1 4

Table II. *Intensity of the 4 and 5 proton signals as a function of storage time at 65°C*

The 9-proton signal intensity = unity

Time (h)	Rel. 4 and 5 proton signal intensity
0	1.65
0.33	1.50
0.67	1.19
1.0	1.14
1.33	0.68
1.67	0.56
2.0	0.50
2.5	0.27

DISCUSSION

As regards the spectrum of pure acridine orange, one great problem is to decide the nature of the two unequivalent methyl components. The following points may be discussed.

1. *The two rings to which the dimethyl amino groups are attached are non-equivalent.* If this was the explanation the proton numbers in the two components would have been of equal integrated intensities. This is not the case.

2. *One component is associated with the chloride the other with the base of the stain.* If this

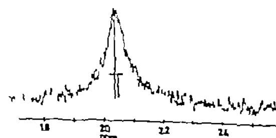


Fig. 4. Methyl line of acridine orange showing the two components with virtually no separation between them. The vertical lines are drawn through the centers of the narrow and broad components.

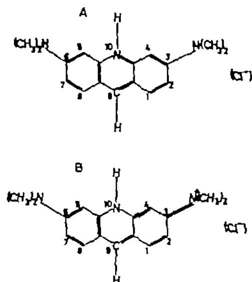


Fig 1 Formulae for the two isomers of acridine orange (without $ZnCl_2$). In structure A, the N at position 10 carries the charge, in structure B the N attached t position 3 carries the charge. Each of these structures may be present as chloride or hydroxide Numbering according to Graebe.

Table I. *Calculation of expected shifts and intensities of ring protons in acridine orange*

References PSB = Pople Schneider & Bernstein, 1959; CD = Corio & Dailey 1956.

Proton no.	9	1 and 8	2 and 7	4 and 5	Ref.
Anthracene	−3 7	−3.3	−2.7	−3.3	PSB
Corr for $N(CH_3)_2$	—	+0.3	+0.8	+0.8	CD
Corr for N_{10}	—	+0.1	+0.4	+0.8	CD
Corr for pyridine ring	+0.7	—	—	—	PSB
Calculated	−3 0	−2.9	−1.5	−1 7	
Intensity	1	2	2	2	

RESULTS

1 *Assignments of spectral lines*

The complete proton NMR spectrum of acridine orange in heavy aqueous solution is shown in Fig. 2. Ring-bound protons show up to the left of the aqueous line with the shifts −2.44 −1 92, −1 40 and −0 32 ppm. The methyl protons occur at 2.05 ppm to the right of the aqueous line. The NH proton is expected to be in the vicinity of this shift (Meyer Saika & Gutowsky 1953) in fact it may be very broad or split by ^{14}N into a triplet. In the present case it could not be identified. In order to perform the assignment of the ring protons the spectrum was integrated (Fig 2) Calibrating the scale with the 12 methyl protons, one proton was found at −2.44 ppm, two protons at −1 92 ppm and two protons at −1 40 ppm. The proton line at −0.32 ppm showed variable intensity as will be discussed in detail below The maximum observed value in relation to the −2.44 ppm line was $1\ 6 \pm 0.1$

Assuming that the molecule is symmetrical (A in Figs. 1 and 5) there should be only one proton at position 9 with an integrated intensity of 1 Thus this proton might have the lowest shift. This is also supported by calculation of the shifts from anthracene shifts (Pople Schneider & Bernstein 1959) using the substituent effects given by Corio & Dailey (1956) The calculations are given in Table I and Fig. 3 which show the expected order of lines. The calculations may well be uncertain to some tenths of a ppm

The lines at −1 92 and −1 40 ppm appear as doublets with a coupling constant of 9 Hz. In order to decide if there was a coupling between them the −1 92 line was irradiated. There occurred a collapse of the −1 40 doublet, indicating that the doublets must originate from neighbour ing protons in the rings. Thus it seems safe to conclude that the −2 44 line arises from proton 9 the −1 92 line from protons 1 and 8 the −1 40 line from protons 2 and 8 and the −0.32 line from protons 4 and 5

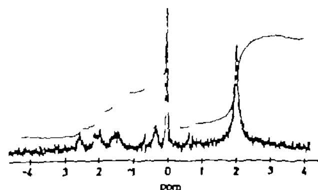


Fig 2 NMR absorption spectrum of 3.3% acridine orange in 99.5% D_2O The ppm scale has its zero point at the aqueous line The upper curves are integral curves.

2. *Behaviour of the −0.32 ppm line*

As mentioned, this line showed somewhat lower intensity than expected. If a sample, dissolved some days earlier was investigated the line had

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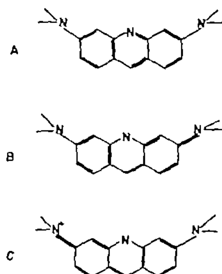


Fig 5 Simplified formulae for the three possible resonating configurations of the acridine orange molecule.

were the case there would be, within a range of three molar equivalents, a titration point at which both fractions were equal. Such a titration point has not been found, see Part II of this series (Höglund Joelsson Ingelman-Sundberg & Odeblad, 1972)

3 *One component is associated with structure A in Fig 1 the other with structure B* This would imply some pH dependence, which has been found. However structure B with a double bond to the dimethyl amino groups should have the broader line (less mobile methyl groups due to hindered rotation of the $N(CH_3)_2$ group) and this fraction should be equal to or less than 0 5 which does not agree with the experimental findings.

4 *The broad component may be due to unresolved partial lines arising from spin coupling with ring protons* Spin decoupling experiments failed, however *to disclose any narrowing of the* broad methyl component.

5 *The splitting into two components is connected with the zinc-acridine orange binding in a way presently not understood*

6 It has been discovered (Höglund & Odeblad 1971) that acridine orange has a complex electron spin resonance spectrum. *The unpaired electron may therefore distribute over the stain molecule in such a way as to broaden some of the methyl protons but not others*

Apparently the question how two methyl components arise cannot be answered at present but must be made the subject of coming investigations.

If the acridine orange molecule were asymmetrical *as in Figs 1 or 5 (structure B)* the protons at positions 5 7 and 8 would belong to an aromatic ring and the protons at positions 1 3 and 4 to an non-aromatic ring. An aromatic ring has more negative shift than a non-aromatic ring, due to the induced diamagnetic current of the π-electrons (Pople 1956) If however the structures 5B and 5C are in resonance with each other *with short persistence time* the whole system turns out, on average to be symmetrical. A slight degree of asymmetry with long persistence time may however exist below the NMR detection limit (a few per cent) The proton NMR spectrum of acridine orange has been studied previously by Kokko & Goldstein (1963) and Blears & Danyluk (1967) Their reported shifts of the ring protons *differ considerably from our measured shifts. In* the first-mentioned paper dimethylsulfoxide was used as solvent which may at least in part, explain the differences. Blears & Danyluk reported a concentration-dependent shift of all ring proton lines towards lower shift at higher dilution in D_2O relative to an internal standard. If their data are extrapolated to our concentration range *the difference may be somewhat reduced.* The presence of $ZnCl_2$ in our samples may also be of some importance.

The exchangeability of the protons in positions 4 and 5 although its significance is not known, may be of some importance in the interaction between stain and tissue compounds.

The existence of two methyl compounds must always be taken into account when considering the binding of the dye on biological material.

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CONCENTRATION OF ANTITHROMBIN III DURING COMBINED AND PROGESTOGEN-ONLY ORAL CONTRACEPTIVE TREATMENT

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Abstract Oral contraceptive treatment with a low daily dose of Norethindrone did not lower the Antithrombin-III level in serum. This was in contrast to what was found in subjects taking estrogen–progestogen agents.

The inhibition of coagulation and fibrinolysis are important factors for maintaining the normal haemostatic balance Antithrombin III (At-III) an α_2-globulin with a molecular weight of about 65 000, is reported to be responsible for about 70% of the progressive antithrombin activity in blood (1) Patients with congenital At III deficiency have an increased tendency to thromboembolic disease (4) Fagerhol et al (6) found a reduced At-III concentration in both plasma and serum of women taking combined oral contraceptive agents while there was no alteration in those on progestogen-only pills Howie et al (8), who measured the At III activity in serum, reported similar results On the other hand, Hedlin & Monkhouse (9) found no alterations in subjects on combined agents

This report concerns the At-III level in a group of women prior to and after 3 weeks of daily treatment with a low dose of a progestogen of the 19-nortestosterone type The results were compared with those found among subjects who had used combined or progestogen-only contraceptives for a longer period of time.

SUBJECTS AND METHODS

Subjects

The following three subject groups were studied.

Group A consisted of 30 regularly menstruating women aged 17 to 34 years (mean 26.5 years), who supplied blood samples prior to medication and after 3 weeks of treatment with 0.3 mg daily of Norethindrone) (17α-ethynyl-17β-hydroxyoestr-4-en-3-one, hereafter called NET).

Group B consisted of 37 women aged 20–39 years (mean 27.2 years) who had taken continuously NET 0.3 mg daily for a period between 11 and 28 months.

Group C consisted of 61 women aged 13–36 years (mean 23.7 years) who had taken cyclically various combined oral contraceptive agents for a period between 9 and 32 months.

Methods

Blood was collected into untreated glass tubes. The blood samples were kept at room temperature for 1–2 hours before the serum was pipetted off and stored at −20°C until testing The At-III was measured according to a slight modification (5) of the technique of Mancini et al. (15) The concentration was expressed in per cent of the concentration in a mixture of equal volumes of sera from 500 apparently healthy blood donors.

RESULTS

During treatment with NET the mean At-III was slightly higher than before treatment (Table I) but the difference was not statistically significant ($p > 0.05$).

Table II shows that during treatment with combined agents (Group C) the mean At III level was lower than that in the controls (Group A before treatment) and also lower than among women on NET (Group A on treatment + Group B). Both differences were statistically significant ($p < 0.001$).

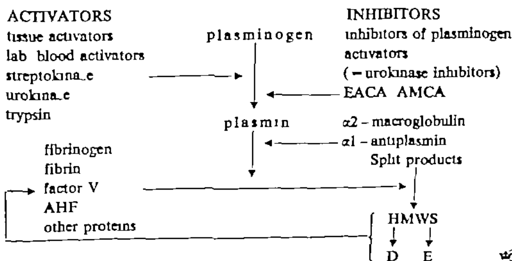
There was a tendency for subjects taking combined agents with a higher dose of estrogen to show a stronger depression of the level of At

Gynaecologists

Menorrhagia may be caused by an increase in local fibrinolytic activity Cyklokapron reduces menorrhagic haemorrhages by an average of 50 %

Women with average menstrual blood losses of over *80 ml* have higher concentrations of plasminogen activators in the endometrium than those with lower blood losses. The resultant increase in local fibrinolytic activity is inhibited by Cyklokapron The recommended dosage of Cyklokapron in menorrhagia is 1 g 3–6 times daily for 3–6 days With a dosage of 3 g daily Nilsson and Rybo noted reductions in bleeding of 38 % compared with control cycles With twice this dosage bleeding was reduced by 51 %. None of the 36 patients participating in the trial were obliged to discontinue treatment as a result of side-effects.

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Table I. *The At III level before and during use of Norethindrone (Group A)*

	Mean At III concentration (±S.E.M.)	
No. of subjects	Before treatment	After 3 weeks of treatment
30	105±[illegible].0	108±2.9

Table II. *The At III level in women on Norethindrone compared with that in women on combined agents*

		At III concentration	
Group	*n*	Mean	S.E.M.
Controls (A)	30	105.0	[illegible].0
Norethindrone (A+B)	67	106.7	1.3
Combined agents (C)	*61*	*92.3*	*1.7*

III than those taking drugs with a low estrogen content.

DISCUSSION

There seems to have been no previous report on the effects of treatment with 19-nortestosterone derivatives alone on various factors of importance for the coagulation mechanism. As such substances are included in most commercially available oral contraceptive agents, the present results are of some interest. It ought however to be noted that the amount of active substance employed in progestogen-only pills is lower than that in most combined drugs.

The present results support previous suggestions that the estrogenic component of contraceptive agents is responsible for the changes of the level of At III (6, 8). An increased incidence of thrombo-embolic disease has been reported among women on combined oral contraceptives (7, 10). This may partly be due to changes in the hemostatic balance as reflected by a reduced level of At III.

Our finding that NET does not lower the At III level is in accordance with some earlier studies of the influence of low-dose progestogen pills on the hemostatic system. Poller et al. (18) and Bloom et al. (2) reported that women on low doses of progestogens had normal levels of factors VII and X. Nilsson et al. (17) found unchanged levels of factor X and of the concentration of fibrinogen and plasminogen, and Bolton et al. (3) reported normal electrophoretic behaviour of the blood platelets. In contrast all these authors found that women taking combined oral contraceptive agents showed alterations of the above-mentioned parameters.

Although the clinical significance of the changes found in the present study are incompletely understood, it must be considered advantageous to use a contraceptive drug that does not induce alterations in the coagulation mechanism. This is further stressed by the fact that the low doses of NET, in contrast to estrogen-containing drugs, have no or only minor effects on carbohydrate mechanism (12), lipid metabolism (14) and liver function (11). This must however be weighed against the circumstance that the frequency of bleeding irregularities and the pregnancy rate are higher than during use of combined oral contraceptive agents (13, 16).

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INTRAVENOUS GLUCOSE TOLERANCE, INSULIN RESPONSE, FASTING BLOOD GLUCOSE AND SERUM INSULIN DURING SHORT TERM ADMINISTRATION OF A COMBINED ORAL CONTRACEPTIVE

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Abstract. A series of 25 healthy women, 13 of whom had tendency to diabetes, was studied for 5 consecutive menstrual cycles, with regard to the effect of combined oral contraceptive containing 0.5 mg norgestrel and 0.05 mg ethinyloestradiol (Eugynon®) on the carbohydrate metabolism. The following tests were performed on these women at midcycle in the cycle prior to, in the two cycles during, and in the second cycle after administration of the drug. Fasting blood sugar, glucose tolerance, fasting insulin and insulin response to glucose injection. The drug was administered daily from the fifth to twenty-fifth day of cycles two and three. Thus each subject served as her own control.

The statistical analysis of the results showed no alteration in the fasting blood sugar or difference between those with diabetic tendency and those without. Glucose assimilation was significantly improved during the second cycle of administration and the follow-up cycle, this was not dependent on the tendency to diabetes. The women with tendency to diabetes had significantly slower assimilation than those with no such tendency. However, the difference remained constant throughout the period under study. The results of the fasting insulin test remained unchanged during the investigation, no difference was seen between the two groups of women, those with the tendency to the disease and those without. The insulin response test showed slightly higher values during the two cycles of administration, this increase was significant, and was seen to occur in both groups. The women with diabetic tendency had slightly higher values in the insulin response test than those of the women belonging to the other group, the difference was not significant except in the second cycle of drug intake.

Following the observation of Wahne et al. (23) in 1963 that a change can occur in glucose tolerance in women under treatment with oral contraceptives towards the values found in diabetic women, numerous studies have appeared of carbohydrate metabolism during the use of oral contraceptives. In general, the results of these various studies have been contradictory and have not permitted of any collective evaluation. This was pointed out by Spellacy (17), Beck (2), Andrews (1) and also in an editorial article in The Lancet (5). Probably the most important cause of this disparity has been the use of different oestrogens and gestagens and also the varying dosages given.

The ideal oral contraceptive should be able to produce reversible protection against pregnancy and at the same time have no side-effects. The problem of the combined contraceptive "pill" and its effect on carbohydrate metabolism has not been solved as yet, but the answer should presumably be sought in the selection of the correct oestrogen and gestagen in the correct dosage, so that the ideal "pill" will cause the least possible alteration to carbohydrate metabolism. With this in mind, we have studied certain parameters of carbohydrate metabolism during short term use of a combined oral contraceptive in common use at the present time. Part of the material employed in this study has also been used in a parallel study of certain parameters of lipid metabolism.

MATERIAL AND METHODS

The series consists of 25 women who consulted us with a view to commencing oral contraception. Their ages varied between 20 and 39 years, average 27. These women were divided into two groups, those being classified as having a tendency to diabetes (group D) and those without this tendency (group N). The criteria used to select those classified as having a diabetic tendency were: Diabetic glucose metabolism during an earlier pregnancy, the birth of a child ≥ 4 500 grams, increase

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changes occurring during the period of the investigation.

The results of the serum insulin test can be seen in Fig. 4 where the mean values for the whole series and for the two groups D and N are illustrated in four diagrams, one for each of the four sets of glucose tolerance tests. The statistical analysis did not demonstrate any changes for the series as a whole for the fasting insulin tests during the period under study and the variance analysis showed no difference between those with and those without a diabetic tendency The values for the tests after injection of glucose were significantly higher for the series as a whole 30 min after the injection in cycle no 2 ($P < 0.01$) and also for those 10 min after injection in cycle no. 3 ($P < 0.05$). The variance analysis on these results showed a significant difference between those with diabetic tendency and those without for the values obtained 60 min after injection ($P < 0.025$) but not for the values at 10 and 30 min.

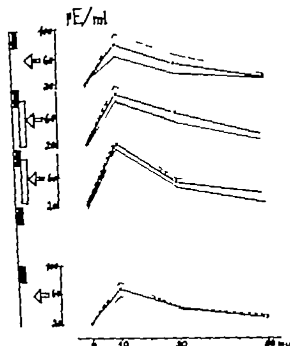


Fig 4 Fasting insulin and insulin response to I. glucose injection. The figure consists of four diagrams, placed one above the other one for each of the four tests. Mean values (Symbols are the same as in Fig. 2.)

DISCUSSION

Spellacy (17) states, in a survey of the literature on oral contraception and carbohydrate metabolism, that collective evaluation of the results is difficult owing to differences such as: the composition of the patients studied, the duration of administration of the drug prior to testing, the time at which the tests are performed in relation to the menstrual cycle, the parameters used for evaluating carbohydrate metabolism, and probably the most important cause of all, a difference in the oestrogens and gestagens and the combination and dosage of these.

In the present study the parameters used were determined prior to, during and after administration of the drug, thus permitting each individual woman to act as her own control. The series was divided into two groups, those with and those without a tendency to diabetes. The time at which the tests were performed in relation to the menstrual cycle was constant throughout the whole trial. The same hormonal drug was administered to all the women, also the dosage and period of administration was the same for all those participating in the study The intention in planning the study in such a manner was to ensure that it would be possible to evaluate the effects of the contraceptive drug on parameters of carbohydrate metabolism used in the study and to see whether tendency to

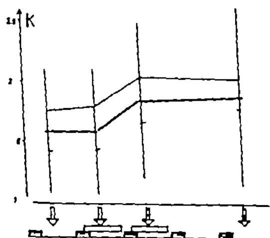


Fig. 3. I. glucose tolerance test. Mean values of the calculated K-values of the four tests. (Symbols are the same as in Fig. 2.)



Fig 1 Plan of the investigation. Hatched areas, menstruation clear areas, hormone administration arrows, tests.

in weight > 0.0 kg during a previous pregnancy hereditary disposition to diabetes. If the woman fulfilled one or more of these criteria she was included in group D All the women were healthy and had no signs or history of any endocrinological disease, and none of them were under treatment with any kind of drug. They were all instructed before entering the study to maintain their normal diet. Those admitted to the investigation had regular menstruation and a minimum period of 3 months had passed since their last pregnancy Apart from the above no selection of the women was made for the study There was no statistical difference between groups D and N with regard to age and parity

The women were studied for a duration of five menstrual cycles, tests were performed four times during the period of observation, namely at midcycle in cycles 1 2, 3 and 5 (see Fig. 1). An oral contraceptive (0.5 mg DL-norgestrel and 0.05 mg ethinyloestradiol) was given daily from the 5th to 25th days of cycles 2 and 3

The tests included determination of the fasting blood sugar and i.v glucose tolerance (ivgt) on all 25 women. In addition the fasting insulin and insulin response to ivgt were determined on 17 of the women (9 in group D and 8 in group N) These tests were carried out in the morning after the women had fasted for 12 hours. Blood for the fasting blood sugar test was removed from an ear and from a cubital vein for the determination of the serum insulin. Fifty ml of 50% glucose solution were injected i.v for the glucose tolerance test and blood withdrawn from the ear every 10 minutes for the following hour The glucose level was determined from these samples. Similarly blood was withdrawn from vein after 10, 30 and 60 min following the injection for the determination of the serum insulin.

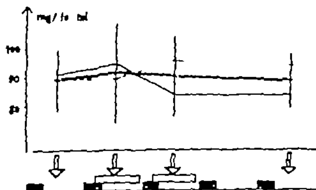


Fig 2 Fasting blood glucose Mean values of the four tests. Thick line, total series; thin line, no tendency to diabetes mellitus; dotted line tendency to diabetes mellitus.

The determination of the glucose content of the blood was carried out by the Central Laboratory of the Odense University Hospital ("Autoanalyzer technicon, N-method, file N-..). The serum insulin determinations were performed by the Farmakologiska Institutionen, University of Lund, Sweden (according to the method of Hedin).

Calculation of the k values of the glucose assimilation was performed after transfer of the blood sugar values to semilogarithmic paper using the formula.

$$k = 2.30 \times \frac{\log C_1 - \log C_2}{t - t} \times 100$$

where C is the blood sugar concentration at the 10 min withdrawal, C the concentration at the 60 min withdrawal and t and t_2 10 and 60 min respectively

The statistical analysis of the material, which was carried out at the EDB Centre, Odense University Hospital, included an evaluation of the series as a whole The individual variations were eliminated by using Student's t-test the method of paired comparisons. In addition a two-way analysis of variance was carried out in order to evaluate the changes occurring during the test period and the difference between the two groups, those with and those without a diabetic tendency

RESULTS

The mean values and standard deviation for the fasting blood sugar are shown in Fig. 2, for the series as a whole. The figure also shows the mean values for each of the groups, D (diabetic tendency) and N (no diabetic tendency) The statistical analysis showed no significant changes between the values in cycle 1 and those for the tests performed during hormonal administration or of the final test carried out after the cessation of drug intake. Neither was any difference found by variance analysis between the four tests carried out, nor any significant difference between the two groups.

The results of the intravenous glucose tolerance tests can be seen in Fig. 3 these are shown as the mean values and standard deviation of the calculated k values for the series as a whole and the mean values for the two groups (D and N) No change was found for the series as a whole between the test prior to drug administration and that carried out in cycle no. 2, but significantly higher values were found in the test performed in cycle no. 3 ($P < 0.025$) and during the follow-up cycle ($P < 0.025$). The variance analysis demonstrated a significant difference between the women with a diabetic tendency and those without ($P < 0.001$) but no difference between the

synthetic gestagens (2) thus a rebound effect may occur

Studies of the serum insulin showed that no change occurred in the fasting values during the administration of the hormone or after its administration, neither was there a difference between those with a tendency to diabetes and those without. A number of studies are available of the fasting serum insulin during the use of a combined contraceptive. The fasting insulin level as found to be both unchanged (11 18 20, 21 25) and increased (9 19 26).

For the series as a whole the insulin response to an i v glucose injection was greater in the two tests carried out during the administration of the drug, and unchanged in those performed after cessation of the hormone, in relation to the tests performed during the control period prior to the drug being given The group with a diabetic tendency showed a greater response than the other group in all four tests. However the difference was only significant in the second cycle during which the drug was administered and then only for the samples withdrawn 60 min after the injection of glucose The changes ran a parallel course for both groups throughout the period of the study Both an increased (11 18, 19 20 25 26) and an unchanged (11 21) response have been reported in the studies that have been published on the insulin response to an i injection of glucose during the administration of an oral contraceptive The importance of the tendency to diabetes has only been studied in a few publications In two studies it was found to be of no importance (19 20) however in the latter study a greater response was seen in women who had given birth to children having an abnormally large birth weight The number of patients in the present study does not permit evaluation of this factor as only three of the omen in group D had given birth to children ha ing birth weight of more than 4 500 grams There re only few studies published dealing ith serum insulin levels immediately after stopping the administration of oral contraceptives. Some hav shown an unchanged response (21 25) and others increased alues (11) in relation to both the control period prior to the use of the drug and the period during which it was administered.

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I am indebted to Professor Karl Kristoffersen, Odense, Professor Jørgen Fabricius, Odense, and Docent Claes Rerup, Lund, for help and advice in performing the study and to the Schering A/S, Ballerup, for supplying the tablets.

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diabetes had any influence on these results. On the other hand this planning does not permit the study to be used for evaluation of the general effects of all oral contraceptives on carbohydrate metabolism or the effects following prolonged use of the drug. For these reasons the following discussion will be confined in general to studies in which similar parameters have been used.

This study has shown that no changes occur in the values of the fasting blood sugar during hormonal administration also that there was no difference between the values obtained in the two groups, those with and those without a tendency to diabetes. The results of many studies have been published on the effect of oral contraception on the fasting blood sugar in the majority of them there was no change in this parameter (3 11 14 15 18 19 21 24 25) an increase in the values of the fasting blood sugar for a number of test subjects was reported by a single author however no control studies had been made during a period free from drug intake in this study (7)

Increased glucose assimilation was found for the series as a whole and for both groups (D and N) during the second cycle of administration and following the cessation of the drug administration this test was performed using i.v glucose tolerance. The assimilation in the group with a diabetic tendency was lower than in the other group throughout the whole period under study The changes in assimilation were parallel Numerous studies have been published showing the results of ivgt during the administration of an oral contraceptive but there is no agreement reduced glucose assimilation was reported in a number of them (4 8 11 13 14 15 16, 18 24 25) and unchanged assimilation in others (3 13 14 16 19 20 21 26) The reduction should occur in particular in women predisposed to diabetes according to some studies (2, 8 14 15 16 22 24) This study has been unable to confirm the above A diphasic course with a primary reduction followed by a secondary increase in assimilation has been found by some investigators (13 15)

A single study employing the same hormonal contraceptive as used in the present investigation has been reported by Jung (11) In her study ivgt showed a significantly reduced assimilation during the second cycle of administration while the values in the second cycle following discontinuation of the contraceptive were similar to those obtained in the control period prior to the drug therapy The plan of this study was almost identical to the plan of the present investigation the most important difference being that Jung's tests were performed somewhat later in the cycle (20th and 22nd day) than in the investigation reported here. This difference in the time of sampling may possibly explain the discrepancy between the results, because it has been found that glucose tolerance varies throughout the menstrual cycle with a maximum around midcycle (6 10) and that this cyclical variation ceases on the administration of hormones suppressing ovulation (6)

An increase similar to that observed in the present work, in glucose assimilation as demonstrated by ivgt during the administration of an oral combination contraceptive has only been reported once (4) In that investigation a combined contraceptive containing 1 0 mg of norethisterone and 0 05 mg of mestranol was used. It is noteworthy that if the dosage was doubled the tolerance fell. The glucose tolerance tests were carried out on the same day in relation to the cycle in both the control period prior to administration and during the use of the drug however the exact day is not mentioned.

Very few studies have been published showing the results of ivgt in the period following the use of an oral contraceptive. Those that have been reported demonstrated a reduced tolerance during the administration of the drug and a return to normal some 2 or 3 months after the drug had been stopped (8 11), also that if normal tolerance was found during the use of the drug then this remained normal after the hormone had been discontinued (21)

A persistent increase in glucose assimilation in the second cycle after cessation of the hormone as demonstrated in the present study does not appear to have been reported previously It is difficult to imagine exactly how this can be explained from the insulin response tests carried out during the same study which showed a return to normal of the increased insulin response demonstrated in the administration phase It is possible that this can be caused by a change in peripheral sensitivity to insulin It has been demonstrated that the peripheral sensitivity to insulin is reduced by the administration of some

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FIBRINOLYTIC ACTIVITY OF VEINS IN THE PUERPERIUM

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Abstract. The content of fibrinolytic enzymes in the vessel walls in the puerperium was determined histochemically in biopsy specimens of hand veins and the release of such enzymes into the blood stream was studied after stimulation by venous occlusion of the arms. The local response of the fibrinolytic activity in the blood to such venous occlusion, which is known to be decreased during pregnancy was almost normal as early as two hours post partum. The fibrinolytic activity in the vessel wall was found to be normal on the 2nd–5th day post partum. The early return of the fibrinolytic activity in the blood and in the vessel wall is probably due to loss of the influence of placental hormones and perhaps also to the loss of a placental inhibitor of fibrinolysis.

In the course of pregnancy the fibrinolytic activity of the blood successively decreases and reaches very low levels at term (2, 3, 5, 12, 18, 26). During labour it rises a little (33). After delivery it soon recovers its non-pregnant level (2, 4, 27). It has recently been shown that the content of fibrinolytic enzymes in the vessel wall as determined histochemically is low during pregnancy and that their release even when stimulated by venous stasis, successively decreases, and is often barely demonstrable at term (34). The cause of this depression of the fibrinolytic activity of blood and in vessel walls during pregnancy is far from clear.

This paper concerns a study of the fibrinolytic activity in the blood and in the vein walls in the puerperium. The fibrinolytic activity in the vein wall was determined histochemically in biopsy specimens of veins (cf. 34) and the local fibrinolytic activity of the blood was studied after venous occlusion of the arms (23). The investigation was extended to include determinations of plasminogen and some inhibitors of the fibrinolytic system.

MATERIAL AND METHODS

The clinical material consisted of 100 apparently healthy volunteers in the puerperium following normal pregnancy and normal delivery of the child and the placenta. The local fibrinolytic activity after venous occlusion of the arms was studied two hours and 1, 2, 3 and 4–5 days after parturition. In 29 of them biopsy specimens of hand veins were obtained on the 2nd, 3rd or 4th–5th day.

40 non-pregnant healthy women in fertile age served as controls. Venous biopsy specimens were obtained from 21 of them.

Venous stasis was produced by placing a sphygmomanometer cuff around the upper arm and inflating it to a pressure midway between the systolic and diastolic blood pressure, for 20 min. Blood samples for the determination of the fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates (19) were drawn from an antecubital vein before the cuff was inflated (spontaneous fibrinolytic activity), and again immediately before it was deflated.

Venous biopsy specimens, about 0.5 cm long, were obtained from the hand under local anaesthesia. The fibrinolytic activity was demonstrated with the histochemical method of Todd (29), as modified and graded according to Pandolfi et al. (21).

The vessels were cut on an International Harris cryostat in sections 8 μ thick and collected on pre-cleaned glass slides. Four slides were prepared for each sample. The sections on each slide were covered with 0.06 ml of fibrinogen (bovine fibrinogen essentially prepared according to Blombäck (6) in a concentration of 1% in phosphate buffer, pH 7.8 ionic strength 0.15) and of 30 μl thrombin (Topostasin 20 NIH units ml unbuffered saline). The fibrinogen-thrombin mixture was spread over an area of 10 cm² in order to obtain a fibrin film about 0.07 mm thick. To stabilize the fibrin film, the slides were left at room temperature (21–24°C) in a moist chamber for 30 min. One of the slides was fixed immediately afterwards in formalin while the remaining 3 slides were transferred to another moist chamber at 37°C and incubated for 10, 20, 30 min, respectively after which they were fixed in formalin. The fibrin slides and the sections were stained with Harris haematoxylin. Fibrinolysis was re-

Fig 1 Fibrinolytic activity f hand veins in 29 puerperal patients and in 21 non-pregnant women in f rtile age Each dot denotes one patient. (Histochemically determined. Arbitrary units.) No significant difference between the groups.

flected by clear lytic areas in the fibrin film at the site of fibrinolytically active cells. Three fai ly distinct grades of fibrin digestion were recognised, namely *grad I microscopical punctate areas in most of the sections; grade II* gross lytic areas of irregular outline and some times confluent, *grad III* dissolution of most o all the fibri in contact with the sections.

A grade I slide was allotted 1 point a grade II slide, points, and a grade III slide, 3 points. The total number f points scored by the set of 4 slides was taken as a measure of the fibrinolytic acti ty of the sample

Other fibrinolytic studies. Plasminogen. Immunological method. Blood collected with epsilonaminocaproic acid (EACA) (9 11) Normal range: 70–130%.

Inhibitors of plasminogen activatio by u okinase (urokinase inhibitors) Clot method (22). Normal range 60–140%

A tiplasmin. Caseinolytic method (9 4). Normal range 400–600 ACU/ml.

Alpha₂-macroglobulin. Esterolytic method (10) Normal range 80–130

Fibrinogen-fibrin degradation products (FDP) Immunochemical method Blood collected with thrombin and EACA (17). This method will not demonstrate FDP in normals, e. < 5 µg ml.

*Statistical methods. The indi idual groups were com*pared by Student t-test The difference in th fibrinolytic activity of the eins as analysed by the Wilcoxon rank sum test.

RESULTS

The values found for the fibrinolytic activity in the walls of the biopsy specimens of the veins are given in the figure. The values did not differ significantly between the puerperal groups or between these groups and the non-pregnant women.

The spontaneous fibrinolytic activity and the response of the fibrinolytic activity to venous occlusion are given in the table. Only the results of tests with resuspended euglobulin precipitate are presented the tests with plasma having given similar results. The response of the fibrinolytic activity to venous occlusion had nearly returned to normal within two hours of parturition ($0.05 > p > 0.01$) It then increased and on the 4th–5th day it was somewhat above normal but the increase was not significant ($p > 0.05$)

Determinations of plasminogen and the inhibitors of the fibrinolytic system are given in the table. Plasminogen was increased, but by the least amount 2 hours after parturition, and it did not return to its non pregnant level during the period covered by the investigation Neither did alpha$_2$-macroglobulin or antiplasmin. Two hours after parturition the inhibition of plasminogen activation by urokinase was just below the normal mean and rose significantly to the 4th–5th day ($p < 0.01$)

FDP (5–20 µg/ml) were demonstrable in 47% 2 hours after parturition. This frequency gradually fell with time.

DISCUSSION

There is convincing evidence that the endothelium of certain vessels, especially veins, contains activators of fibrinolysis, which are continuously liberated to the blood stream, where they maintain the spontaneous fibrinolytic activity of the blood (20) This spontaneous fibrinolytic activity (2, 3 5 12, 18 26) as well as the fibrinolytic activity stimulated by venous occlusion (34) has been found to decrease successively during pregnancy and at term it is sometimes barely demonstrable Also the content of fibrinolytic activators in the vessel wall has been found to be low in pregnancy (34)

In the present investigation the local fibrinolytic activity stimulated by enous occlusion was nearly normal ($0.05 > p > 0.01$) within 2 hours of parturition and reached a normal level on the following day The fibrinolytic activity in the vessel wall of vein biopsy specimens was normal within 2–5 days. FDP were found in 47% 2 hours after parturition. This frequency decreased with time an observation also made by Stiehm et al. (28)

ble I. Mean values and S.D. of fibrinolytic components in 100 puerperal women (20 in each group) and in non-pregnant women

…s after parturition	2 hrs	1 day	2 days	3 days	4–5 days	Non-pregnant
…inogen, …	109±24	124±22	133±26	142±38	134±43	90±12
…nase inhibitors, %	101±30	112±24	126±31	127±28	140±37	111±14
α_2-macroglobulin, …	162±45	144±41	145±35	134±23	141±39	116±21
…plasmin, ACU/ml	779±87	832±60	844±108	845±88	808±101	417±55
…aneous fibrinolytic activity …heated fibrin plates …up. euglob. prec., mm²	20±22	24±29	23±19	23±19	41±28	31±26
…olytic activity on unheated …n plates after venous occlusion …up. euglob. prec., mm²	233±119	312±158	292±126	313±108	356±150	303±102
…(3–20 µg/ml) as % of the whole group	47	43	30	20	30	0

…ter quick return of the blood fibrinolytic ac…ty and the fibrinolytic activity in the vessel …ls is surprising. Astedt (32) compared the local …ponse of the fibrinolytic activity to venous …lusion 1 hour after parturition, in women in …om delivery of the child had been normal but …hom the placenta had been retained, with …t in a series where delivery of both the child …d the placenta had been normal. The local …ponse of the fibrinolytic activity was still low …the women with retention of the placenta but …most normal in the women in whom the delivery …d been normal. These findings suggested that …e depression of the blood fibrinolytic activity …ring pregnancy is due to the presence of the …acenta, and not to that of the foetus. Further…ore, it has been shown in combined organ cul…re that placenta has an inhibitory effect on the …brinolytic activity released from vessel explants …15).

The placenta has been found to contain large …mounts of inhibitors of urokinase-induced fibrino…ysis by Kawano et al. (15) and Åstedt and …Uszynski (1) and they feel that these inhibitors …ight be responsible for the low fibrinolytic …ctivity in pregnancy. But opinions differ on the …rokinase inhibiting effect of the blood in preg…nancy, some authors having found it to be in…creased (7, 16, 25), others normal, or even de…creased (3, 8, 13, 18). In the puerperium the uro…kinase inhibitors have been studied by Shaper et al. (25) who found a gradual return to normal of the elevated pregnant levels. Nilsson and Kull…lander (18), Ygge (30), Bonnar et al. (4) found an increase during the puerperium like the results in this investigation. These observations suggest that the urokinase inhibitors are not responsible for the decreased fibrinolytic activity of the blood. In addition it should be mentioned that the levels of alpha$_2$-macroglobulin and antiplasmin were still raised.

The changes caused by the placenta, in fibrinolytic activity in the blood and in the vessel walls during pregnancy and the puerperium might have another explanation. It has recently been shown that ethinyloestradiol (31) and prednisolone (14) lower the fibrinolytic activity in the vessel walls. The secretion of steroid hormones by the placenta might directly or indirectly act upon the synthesis and release of fibrinolytic activators in the vessel walls. One might imagine that the suppressed release and the reduced synthesis of activators of fibrinolysis during pregnancy can rapidly recover after separation of the placenta and thereby loss of its hormonal influence.

ACKNOWLEDGEMENT

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CYSTINE AMINOPEPTIDASE ACTIVITY IN PREGNANCY

II. *Its Clinical Application as an Index of Placental Function*

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Abstract. Using new method for estimation of cystine aminopeptidase (CAP) activity in maternal serum, serial CAP determinations have been performed in 7 women during normal pregnancy in 7 patients after delivery in 30 patients with pre-eclampsia, in 3 patients with suspected placental insufficiency and in 4 multiple pregnancies. The results are as follows.

The enzyme activity decreased slowly after delivery. The half life of the enzyme in maternal blood was calculated at 3–6 days except in one patient where only 20% of the enzyme activity was lost after 4 days. The decrease of CAP activity in serum after intra-uterine foetal death was also very slow. In patients with uncomplicated mild toxaemia of pregnancy the CAP values were in the normal range. In patients with severe pre-eclampsia the result varied. In one patient with acute severe pre-eclampsia and threatening eclamptic attack the CAP values were high, and this might be explained by the presence of non-specific aminopeptidase activity in serum. In pre-eclampsia with associated retardation of foetal growth the CAP values were rather low with decreasing values prior to foetal death. In patients with pronounced placental insufficiency the CAP values were consistently low whereas high figures were often found in multiple pregnancy.

The observations indicate that determination of CAP in maternal serum might be a valuable parameter in judging the condition of the placenta. However the significance of CAP assays in assessing placental function cannot be established until the method is compared with other placental function tests on the same clinical material.

One of the main problems in modern obstetrics is to confirm the clinical suspicion of placental insufficiency in order to estimate the optimal time and the safest method of delivery for both mother and child. For this purpose estimation of urinary oestriol excretion has been widely used. However this method has some limitations. It necessitates 24 hour samples of urine, the range of normal values is rather wide and great fluctuations in oestriol excretion from day to day are often found in the same patient. Therefore it seems justified to search for other parameters which might be of value for evaluating placental function during the last trimester.

During normal human pregnancy an aminopeptidase named oxytocinase or cystine aminopeptidase (CAP) appears in maternal serum (14). The amount of enzyme increases during pregnancy and reaches a maximum near term (6). The placental origin of the enzyme has been established (8). Babuna & Yenen (1), Josephides & Turkington (4) and Tovey (13) have suggested that determination of the enzyme activity in maternal serum might reflect the functional state of the placenta. The method used for estimation of the enzyme activity in these studies was that described by Tuppy & Nesvadba (14). In studies concerning CAP activity in different tissues, Rydén (8) concluded that aminopeptidase activity from sources other than the placenta could influence the estimation in the maternal serum and give falsely high CAP values. Therefore a new rapid method for estimation of the enzyme activity in the blood of pregnant women which compensated for the nonspecific aminopeptidase activity has been evolved. The method shows a 50-fold increase of the enzyme activity during pregnancy as compared with a 20-fold increase using the original CAP procedure (9). Based on about 700 blood samples a standard curve was constructed of the CAP activity between the 21st and 42nd weeks of pregnancy.

The purpose of the present investigation was

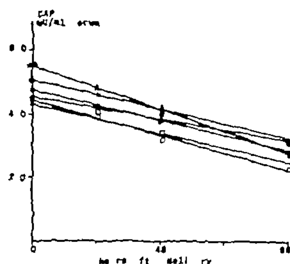


Fig 1 The disappearance of CAP activity after delivery plotted on a semi-logarithmic graph.

to study the clinical usefulness of CAP estimation, with special attention to the problem of placental insufficiency

MATERIAL AND METHODS

Blood samples

About 10 ml of maternal venous blood were taken. The samples were usually stored in a refrigerator and estimations performed within 48 hours. If the interval before assay exceeded 48 hours the samples were kept at −20°C.

The method of cystine aminopeptidase (CAP) assay and the calculation of the enzyme activity was the same as described previously (9).

Selection of patients Normal pregnancy Criteria of normality were: The date of the last menstrual period was certain, The uterine size was compatible with the period of amenorrhoea, The blood pressure was below 140/90 and there was no proteinuria, There was no abnormal bleeding during the pregnancy

Moderate pre-eclampsia. Blood pressure was 140/90 or higher but did not exceed 160/100, there was oedema but no proteinuria.

Severe pre-eclampsia. Blood pressure was higher than 160/100 and there was oedema and/or proteinuria. Patients with proteinuria but with blood pressure between 140/90 and 160/100 were also included in this group.

Possible placental insufficiency Women with disparity between the uterine fundal height and that expected from the period of amenorrhoea.

Miscellaneous group This group includes 2 patients with a previous history of repeated abortions and 4 patients with multiple pregnancy

RESULTS

Maternal blood samples from 7 women were taken at delivery and 24, 48 and 96 hours after delivery. The CAP activity was estimated. The

Table I The disappearance of CAP activity from maternal blood after delivery

Patient	At delivery	CAP activity mU/ml serum (hours after delivery) 24	48	96	$T_{½}$ (days)
1	6.6	5.2	4.2	—	3 days
2	5.0	—	4.0	2.6	5 days
3	4.6	3.8	3.0	2.2	4 days
4	4.4	4.0	3.2	—	5 days
5	6.6	4.8	4.0	3.2	5 days
6	5.6	4.2	3.8	3.0	6 days
7	5.0	4.8	4.8	4.2	—

values were plotted on a semilogarithmic graph (Fig 1) from which could be calculated an approximate elimination rate of the enzyme from the blood after delivery. In two women the half life was estimated to 3 and 4 days respectively, in three women 5 days and in one woman 6 days (Table I). In one woman only 20% of the activity was lost within 4 days. No explanation for the slow decrease in CAP levels after delivery in this patient could be found in the records.

The results of serial CAP assays in normal pregnancy are presented in Fig. 2. All birth weights were within the normal. In 3 patients there was a tendency to decreasing CAP values prior to the onset of labour

Six women with mild toxaemia of pregnancy were followed with serial CAP assays. The results

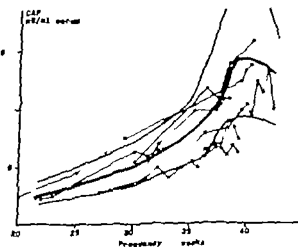


Fig Serial CAP assays in normal pregnancy. The solid line in this and the subsequent figures represents the mean values constructed from about 700 estimations during different stages of gestation. The 10th and 90th percentile lines are also shown.

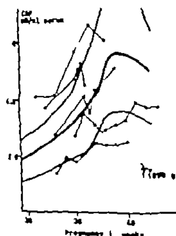


Fig 3 Serial CAP assays in mild pre-eclampsia. In one patient with stillborn infant the cessation of foetal movements is indicated by an arrow. The weight of the infant shown in the figure. Pronounced degenerative changes were found in the placenta.

are shown in Fig. 3. In 5 cases there was an uncomplicated delivery and a normal birth weight. In one case the duration of the pregnancy was uncertain, because the last menstrual period was a withdrawal bleeding due to treatment with hormonal contraceptives. The duration of the pregnancy is calculated from the last menstrual period. The foetal movements ceased 5 days before the patient was admitted to the hospital in labour and the CAP level was very low when the patient came into the hospital. Marked degenerative changes were found in the placenta.

The results obtained in 6 patients with severe toxaemia of pregnancy are presented in Fig. 4. The patient with extremely high CAP values came into the hospital with a threatening eclamptic attack. She was treated with hypotensive agents, sedatives and diuretics, and when the blood pressure was under control labour was induced. Two patients were delivered by cesarean section (C.S.), one patient because of intrauterine asphyxia when labour started, and the patient with very low CAP

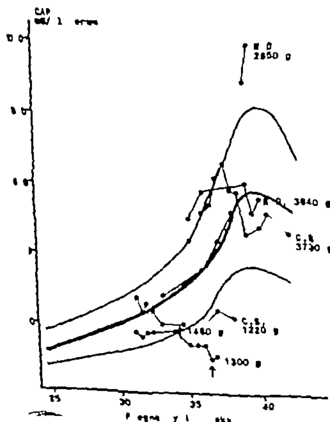


Fig 4 Serial CAP assays in severe pre-eclampsia. The arrow indicates time of intra-uterine foetal death. *N.D.* Normal delivery. *C.S.*, cesarean section. The weights of the infants are given in the figures.

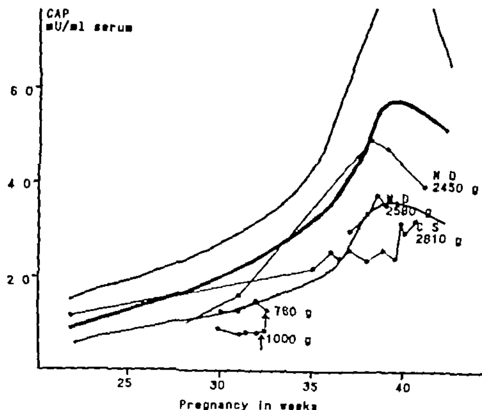


Fig 5 Serial CAP assays in suspected placental insufficiency The arrow indicates time of intra-uterine foetal death. N.D Normal delivery C.S cesarean section. The weights of the infants are given in the figure.

values were delivered because the oestriol values declined Two patients with hypertension proteinuria and with clinical signs of arrested foetal growth had slowly decreasing CAP values during the weeks that preceded foetal death in utero However no further decrease of the CAP value occurred from the day of intra uterine foetal death to the following day

Five women with clinical signs of placental insufficiency were followed with serial CAP assays. The results are shown in Fig. 5 Two women with very pronounced arrest of foetal growth had consistently low CAP values during three weeks preceding intra uterine foetal death. One woman was delivered by cesarean section (C. S) because of intra-uterine asphyxia.

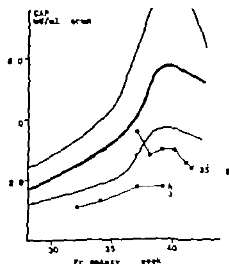


Fig 6 Serial CAP assays in two women with previous history of recurrent early abortion N.D normal delivery

Two women with previous histories of recurrent early abortions (5 and 3 respectively) were followed with serial CAP assays (Fig. 6) The CAP values were rather low and decreased in one case during the last weeks. Labour was induced in the patient with the lowest CAP values, whereas it started spontaneously in the other During delivery there were no signs of intra-uterine asphyxia and the infants were of normal weight.

Three twin pregnancies and one triplet pregnancy were also followed with serial CAP determination The results are shown in Fig. 7 and indicate that multiple pregnancy is often attended by increased CAP values.

DISCUSSIONS AND CONCLUSIONS

Despite the fact that the aminopeptidase specific to human pregnancy has been known for many years, the clinical significance of CAP estimation in pregnancy is still uncertain In recent years many reports have been published concerning

the value of determining the enzyme activity in maternal blood as a test of the functional state of the placenta (1 3 4 5 13) but these studies do not give convincing evidence concerning the usefulness of CAP estimations in clinical practice.

Thus, the studies on the elimination rate of the enzyme in blood after intra-uterine foetal death and after delivery are contradictory. Babuna & Yenen (1) demonstrated a 50% decrease in CAP activity one day after intra-uterine foetal death, whereas Tovey (13) and Melander (6) found a rather slow decrease of enzyme activity after delivery. From their diagrams a half-life of the enzyme in blood of about one week can be calculated. In the present study the half-life of CAP after delivery was found to be between 3.0 to 6.0 days in six women. In one woman the elimination rate was extremely slow and only 20% of the activity was lost within 4 days. Neither was there any abrupt decrease in CAP activity after intra-uterine foetal death in 3 cases which were examined one day after intra-uterine foetal death. The slow decline in CAP activity is explained by the fact that the enzyme responsibility for the activity has a molecular weight of about 300 000, according to Yman (17) and is therefore not excreted in the urine. Klimek & Maloleprzy (5) ha suggested that serial estimations of CAP at intervals of one or several hours might be of importance for evaluating the state of the foetus in utero. The present study does not confirm this conclusion.

The data on CAP in patients with pre-eclampsia is somewhat contrary. In mild toxaemia of pregnancy the CAP values are very similar to those found in normal pregnancy. The same results were found by Josephides & Turkington (4). In the patient where the CAP values were consistently low during the last 3 weeks before delivery no signs of acute asphyxia or of foetal growth arrest were found at delivery. In one patient of this group with intra-uterine foetal death, the duration of pregnancy was uncertain, and no analyses of CAP were performed during the 4 weeks that preceded the death of the foetus. Five days later the CAP activity was extremely low and an analysis performed weeks earlier might possibly have detected the threatening danger of the foetus.

The results obtained in patients with severe pre-eclampsia were variable. In 3 women the CAP levels were very low and in 2 of these patients slowly declining CAP values were found prior to intra-uterine foetal death. One patient with very low CAP values was delivered in the 38th week by cesarean section, and a dysmature baby weighing 1 220 g was born. One patient in this group had very high CAP values on admission to the hospital. She had pronounced hypertension, headache, oedema and massive proteinuria, and was treated for a threatening eclamptic attack. The high CAP values found in this patient might be due to aminopeptidases from other sources or to a massive flow of the specific aminopeptidase activity from the placenta due to damage of the placenta. In a previous study Rydén (8) demonstrated that many organs, i.e. the liver the myometrium, the placenta contain "nonspecific" aminopeptidase activity which are capable of reacting on the same substrate as oxytocinase. This enzyme activity can be demonstrated in the soluble protein fraction in the tissue homogenate. It is therefore probable that this enzyme may leak out of the cells due to lesions in these organs, and thus give false high values of CAP under certain conditions. The results of Müller Hartburg, Nesvadba & Tappy (7) who found

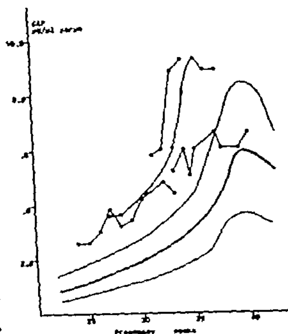


Fig. 7. Serial CAP assays in three twin pregnancies and in one triplet pregnancy (open symbols).

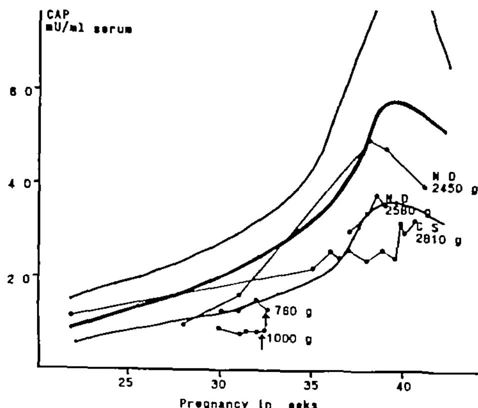


Fig 5 Serial CAP assays in suspected placental insufficiency The arrow indicates time of intra-uterine foetal death. *N.D* Normal delivery *C.S* cesarean section. The weights of the infants are given in the figure.

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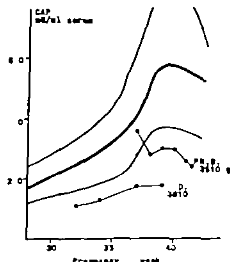


Fig 6. Serial CAP assays in two women with previous history of recurrent early abortion. *N D* normal delivery

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SPERM PENETRATION OF CERVICAL MUCUS AS A CRITERION OF MALE FERTILITY

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Abstract Semen samples ere examined from 51 fertile men, and from the male partners in 283 infertile marriages. In addition to routine analysis, they were tested for sperm penetration through ovulatory cervical mucus, using the method of Kremer ith slight modifications. Comparison f the 2 groups in regard to semen properties showed statistically significant differences between the means for density percentage of living, motile and ab normal spermatozoa, for motility degree, and for sperm penetration No significant difference between the means as found for volume, content of fructose and acid phosphatase in the seminal plasma. In the entire series moderate correlation as found between sperm penetra- test and percentage of motile spermatozoa, motility degree, density percentage of living and percentage of abnormal spermatozoa. A low correlation as found between sperm penetration and volume, content of fructose or acid phosphatase in seminal plasma. The variance of sperm penetration due to regression of the other semen proper ties as only 43%. Sperm penetration had the highest discriminant function coefficient of the tested semen properties. Semen samples from fertile men had high penetration values although other semen properties were sometimes pathological. The sperm penetration test is most valuable supplement to routine semen analyses for male fertility estimation

No in itro test can give exact information about the fertility status of men. In the semen analyses information on the cellular characteristics and the biochemistry of seminal plasma is re- ealed, but there is need for better evaluation of male f rtility Numerous in estigations have been undertaken in order to study the passage of the spermatozoa from the agina through the cervical canal, the cavum uteri and finally through the oviducts to the ovum Many steps still remain obscure In humans the spermatozoa must leave the seminal plasma and migrate into the cervical mucus. Successful penetration varies according to the time in the menstrual cycle (1 17).

Capillary tube methods for measuring sperm penetration in vitro have been described by Botella Llusia (5), Bergman & Gennser (3), Kremer (12) and Carlborg (7). The method of Kremer has been evaluated in our laboratory (20) and found to give reliable results.

The aim of the present investigation was to study the alue of sperm penetration tests for infertility investigations, to compare the results of semen analysis and the in itro sperm penetration for semen samples from fertile men and from male partners in infertile marriages, to find the correlation between the in vitro sperm penetration and the semen quality and to calculate the discriminant function coefficients for the sperm penetration and semen properties

MATERIAL

The investigation concerned semen samples from 334 men, 283 of them from male partners in infertile marriages. In the tables and text this group is called infertile Fifty-one samples ere from men with recently demonstrated fertility either fertile donors for heterologous insemina- tions or ones hose ives had conceived within the past 2 months This group as designated "fertile When several semen samples from the same man ere tested, the mean values were employed in the calculations. The ages of the men varied from 22 to 45 years in the in- fertile group, and from 24 to 39 in the fertile group.

METHODS

Semen analyses The semen samples ere taken by masturbation after at least 3 days of continence, and brought to the laboratory in plastic condom, specially

higher CAP values in blood from patients with liver diseases are in agreement with this assumption. This nonspecific aminopeptidase is unstable in the presence of the substrate, as has previously been shown (8) With the present procedure for estimation of CAP (preincubation for one hour before measurement of the enzyme activity) lower levels of "nonspecific" aminopeptidase in the blood are compensated for If greater amounts of this nonspecific activity are present in serum, the procedure adopted cannot compensate for this activity This is undoubtly a draw-back of the method. Nevertheless in 3 women with severe pre-eclampsia very low CAP values were found which well-reflected the functional state of the placenta. In two patients with intra-uterine foetal death the CAP values were slowly declining during 3 weeks before the death of the foetus, also implicating CAP as a valuable placental function test.

In women with clinical suspicion of placental insufficiency the CAP values were within the normal range in 2 patients and they both delivered normally In a third patient the CAP level was rather low and she was delivered by cesarean section because of foetal asphyxia. Two patients were admitted to the hospital after 30 weeks gestation because of premature separation of the placenta and premature contractions respectively The CAP values were consistently low until intra uterine death occurred. These results also indicate that estimation of CAP can be of value to determine the functional state of the placenta.

However the results in Fig. 6 where one patient with consistently low CAP values and another patient with low and decreasing CAP values were delivered normally with no signs of dysmaturity of the infants, indicate that the results of CAP assays must be interpreted with caution and judged in relation to other placental tests and the clinical history Recently other tests have been evolved in order to assess foetal growth and the condition of the placenta. Measurement of human placental lactogen (HPL) or human chorionic somatomammotrophin (HCS) in pregnant women seems to have limited value due to great individual variations according to Seppälä & Ruoslahti (10) whereas other authors, Spellacy et al. (11) Varma et al. (15) Teoh et al. (12) have published more positive results. Serial measurements of the biparietal diameter by ultrasound for assessing foetal growth seems to be more promising (2 16) The present study implies that determination of CAP in maternal serum can be a valuable parameter in assessing the condition of the placenta However in patients with severe pre-eclampsia the values must be judged with caution as false high CAP values can be obtained. An advantage with the present procedure is that the test is easy to perform and can be used as a simple routine test on out-patients. However the importance of CAP assays in assessing placental function cannot be established until the method is compared with other placental function tests on the same clinical material.

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Table III. *Coefficients of correlation between the various semen properties based on values from the entire material*

	Volume (ml)	Density (mill./ml)	Living (%)	Motile (%)	Motility degree	Abnormal (%)	Fructose (mg %)	Acid phosphatase	Penetration
Volume (ml)	1.00	−0.07	−0.05	0.05	0.04	0.01	0.11	−0.16	0.04
Density (mill./ml)		1.00	0.32	0.43	0.41	−0.40	−0.24	0.21	0.40
Living ( )			1.00	0.66	0.55	−0.51	−0.06	0.07	0.46
Motile (%)				1.00	0.71	−0.56	−0.05	0.09	0.60
Motility degree					1.00	−0.48	−0.10	0.16	0.56
Abnormal forms ( )						1.00	0.10	−0.05	−0.47
Fructose (mg %)							1.00	−0.31	−0.10
Acid phosphatase (IE/ml)								1.00	0.10
Penetration (mm/3 hours)									1.00

The correlation coefficients are given in Table III. For each semen quality the correlation coefficient to penetration is given in the last column. The highest correlations with penetration were found for percentage of motile spermatozoa (r = 0.60) and for motility degree (= 0.56). Moderate correlations were found between sperm penetration and percentage of living spermatozoa (r = 0.46), percentage of abnormal spermatozoa (= −0.47), and density (= 0.40). A low correlation was found between sperm penetration and volume, content of fructose and acid phosphatase in seminal plasma. The highest correlation coefficients found in this work are between percentage of motile spermatozoa and motility degree (= 0.71), and between percentage of living and motile spermatozoa (= 0.66).

When the fertile and the infertile groups were analysed separately, no great differences in the correlation coefficients were found either between the fertile and the infertile groups, or between either one of these and the entire material.

Stepwise regression analysis for all semen samples gave the results which appear in Table IV. The multiple correlation coefficient (R) and the variance accounted for by regression can be seen for each step of the procedure when each variable was added. Only 43% of the variance of penetration was due to regression of the semen properties tested. This value was obtained when percentage of motile spermatozoa, motility degree, percentage of abnormal spermatozoa and density were added. Further addition of variables did not give a higher R. There was a residual variance of 57% which was not due to regression of the semen qualities included in the semen analysis of this material.

When the fertile and infertile groups were considered separately the stepwise regression analysis showed a somewhat higher variance of sperm penetration due to regression for the fertile group (45%) than for the infertile group (37%).

The discriminant function coefficients (L) for each semen property appear in Table V. Penetration had the highest L-value. Percentage of motile spermatozoa and motility degree had lower

Table IV. *Stepwise regression for semen properties in the entire material*

Multiple correlation coefficient (R) and variation due to regression (R^2 100) are given for each step

Variable added	R	R^2 100
Percentage of motile spermatozoa	0.60	37
Motility degree	0.63	40
Percentage of abnormal spermatozoa	0.64	4?
Density of spermatozoa	0.65	43
Percentage of living spermatozoa	0.65	43
Volume of ejaculate	0.65	43
Content of fructose in seminal plasma	0.65	43
Content of acid phosphatase in seminal plasma	0.65	43

Table V. *The discriminant function coefficient* (L) *for the different semen properties with regard to fertility*

Semen property	L
Volume of ejaculate	0.35
Density of spermatozoa	0.02
Percentage of living spermatozoa	0.03
Percentage of motile spermatozoa	0.40
Motility degree	0.39
Percentage of abnormal spermatozoa	0.24
Content of fructose in seminal plasma	−0.02
Content of acid phosphatase in seminal plasma	0.02
Sperm penetration of cervical mucus	1.00

Table I *Mean and standard error of the mean (S E.) for semen properties in the fertile and infertile groups*

Semen property	Fertile group		Infertile group	
	Mean	S.E.	Mean	S.E.
Volume (ml)	3.43	±1.77	3.84	±0.98
Density (mill./ml)	173.07	±13.04	112.55	±6.30
Living (%)	75.11	± 1..6	64.56	±0.93
Motile (%)	55.17	± 1.40	41.34	±0.93
Motility degree	3.11	± 0.07	2.35	±0.04
Abnormal forms (%)	41.37	± 1.56	53.92	±1.01
Fructose (mg%)	368.01	±23.39	414.18	±9.70
Acid phosphatases (I.E.)	40 901.90	±2 865.80	35 770.30	±1 ..33.00
Penetration (mm/3 hours)	34.43	±1.07	19.62	±0.87

med. for this purpose. The semen analysis and the sperm penetration tests were started within 2 hours of ejaculation. The volume of the ejaculate was measured. Sperm density was counted in a Buerker chamber. The percentage of motile spermatozoa and the motility degree were determined by microscopy in a drop of well mixed, fresh semen placed on a slide and covered with cover slip at room temperature. Percentage of motile spermatozoa was estimated to the nearest 5%. The degree of motility was registered numerically 0 = no motile spermatozoa, 1 = motility in loco without forward movement, = slow 3 = rapid, and 4 = very rapid forward movement. The spermatozoal morphology was studied in smears by the method of Moench (16). Supravital staining was performed by the method of Hancock (11). The content of fructose in seminal plasma was determined according to the method described by Mann (15). Acid phosphatase activity in seminal plasma was determined using the technic [illegible] tlined in *Sigma Technical Bulletin 104* (19). The following values for the laboratory findings were considered pathological volume < 2 ml, density < 60 millions ml, percentage of living spermatozoa < 70 percentage of motile spermatozoa < 50 motility degree 0, 1 or 2, percentage of morphologically abnormal spermatozoa ≥ 50, content of fructose < 100 mg 100 ml and acid phosphatase < 20 000 I E. ml.

Sperm penetration test The penetration tests were performed with the technic described by Kremer (12, 13), slightly modified (20). Cervical mucus of ovulatory character carefully pretested and fulfilling certain criteria mentioned in a previous paper (20) was used as test medium. Capillary tubes of inner diameter 0.6 mm and length 41 mm were filled with cervical mucus; the number of tubes obtained from one sample varied from 14 to 42. If not used immediately the tubes of mucus were sealed and stored at +4°C. Storage time did not exceed 3 weeks. Incubation temperature for the tests was 37°C and incubation time 3 hours. The distance which the foremost spermatozoa had moved was measured in millimeters and used as the value for sperm penetration.

Statistical calculations. For all semen properties the mean and the standard error of the mean were calculated. Differences of the means for each quality between the fertile and infertile groups were tested by the ordinary two sample t-test at the 5% level of significance.

Stepwise multiple regression analysis was performed for all semen samples and for each group separately. Sperm penetration was considered as the dependent variable and the semen properties as the independent. The variable with the highest correlation to penetration was first taken into the regression equation, and the others added one by one according to decreasing correlation. For each step the multiple correlation coefficient (R) was determined. The determination coefficient (R^2 100) gives the *percentage of variance due to regression* (6).

For the set of variables the discriminant function coefficients for the 2 groups were calculated in order to detect the variables which were most suitable for *discriminating between fertility and infertility*

Comparisons between distributions were made by the chi square test.

RESULTS

In Table I the mean and standard error of the mean for each semen property in the fertile and infertile groups are given The differences between the means of the semen qualities for the fertile and infertile groups, standard deviations of the differences, and the t values appear in Table II. The fertile group had statistically significantly higher percentage of motile spermatozoa motility degree percentage of normal and living spermatozoa, *density and sperm penetration. For volume content of fructose and acid phos*phatase the differences between the fertile and infertile groups were not significant.

Table II *Difference between the means* ($\bar{d}$) *for semen properties in the fertile and infertile groups*

S_d = standard deviation of the difference, t_{crit} = 1.96. Degrees of freedom = 33.. Level of significance 5

Semen property	$\bar{d}$	S_d	$\bar{d}/S_d$ t
Volume (ml)	−0.41	0.24	−1.69
Density (mill./ml)	60.53	15.81	3.83
Living ( )	10.56	2.30	4.59
Motile ( )	13.83	31	5.99
Motility degree	0.56	0.11	5.09
Abnormal forms ( )	−12.55	2.46	5.10
Fructose (mg %)	−46.16	24.81	−1.86
Acid phosphatase (I E.)	5 134.00	316.00	1.61
Penetration (mm/3 hours)	14.81	2.15	6.89

Significant at the 5% level.

Table III. Coefficients of correlation between the various semen properties based on values from the entire material

	Volume (ml)	Density (mill./ml)	Living (%)	Motile (%)	Motility degree	Abnormal (%)	Fructose (mg %)	Acid phosphatases	Penetration
Volume (ml)	1.00	−0.07	−0.05	0.05	0.04	0.01	0.11	−0.10	0.04
Density (mill./ml)		1.00	0.32	0.43	0.41	−0.40	−0.24	0.21	0.40
Living (%)			1.00	0.66	0.55	−0.51	−0.06	0.07	0.46
Motile (%)				1.00	0.71	−0.56	−0.05	0.09	0.60
Motility degree					1.00	−0.48	−0.10	0.16	0.56
Abnormal forms (%)						1.00	0.10	−0.05	−0.47
Fructose (mg %)							1.00	−0.31	−0.10
Acid phosphatases (IE/ml)								1.00	0.10
Penetration (mm/3 hours)									1.00

The correlation coefficients are given in Table III. For each semen quality the correlation coefficient to penetration is given in the last column. The highest correlations with penetration were found for percentage of motile spermatozoa ($r = 0.60$) and for motility degree ($r = 0.56$). Moderate correlations were found between sperm penetration and percentage of living spermatozoa ($r = 0.46$), percentage of abnormal spermatozoa ($r = -0.47$), and density ($r = 0.40$). A low correlation was found between sperm penetration and volume, content of fructose and acid phosphatase in seminal plasma. The highest correlation coefficients found in this work are between percentage of motile spermatozoa and motility degree ($r = 0.71$), and between percentage of living and motile spermatozoa ($r = 0.66$).

When the fertile and the infertile groups were analysed separately no great differences in the correlation coefficients were found either between the fertile and the infertile groups, or between either one of these and the entire material.

Stepwise regression analysis for all semen samples gave the results which appear in Table IV. The multiple correlation coefficient (R) and the variance accounted for by regression can be seen for each step of the procedure when each variable was added. Only 43 % of the variance of penetration was due to regression of the semen properties tested. This value was obtained when percentage of motile spermatozoa, motility degree, percentage of abnormal spermatozoa, and density were added. Further addition of variables did not give a higher R. There was a residual variance of 57 %, which was not due to regression of the semen qualities included in the semen analysis of this material.

When the fertile and infertile groups were considered separately the stepwise regression analysis showed a somewhat higher variance of sperm penetration due to regression for the fertile group (45 %) than for the infertile group (37 %).

The discriminant function coefficients (L) for each semen property appear in Table V. Penetration had the highest L-value. Percentage of motile spermatozoa and motility degree had lower

Table IV. *Stepwise regression for semen properties in the entire material*

Multiple correlation coefficient (R) and variation due to regression ($R^2 \cdot 100$) are given for each step

Variable added	R	$R^2 \cdot 100$
Percentage of motile spermatozoa	0.60	37
Motility degree	0.63	40
Percentage of abnormal spermatozoa	0.64	42
Density of spermatozoa	0.65	43
Percentage of living spermatozoa	0.65	43
Volume of ejaculate	0.65	43
Content of fructose in seminal plasma	0.65	43
Content of acid phosphatases in seminal plasma	0.65	43

Table V. *The discriminant function coefficient (L) for the different semen properties with regard to fertility*

Semen property	L
Volume of ejaculate	−0.35
Density of spermatozoa	0.02
Percentage of living spermatozoa	0.03
Percentage of motile spermatozoa	0.40
Motility degree	0.39
Percentage of abnormal spermatozoa	[illegible]
Content of fructose in seminal plasma	0.02
Content of acid phosphatases in seminal plasma	0.02
Sperm penetration of cervical mucus	1.00

Table VI. Distribution of the semen samples in the fertile and infertile groups according to sperm penetration extent

Sperm penetration (mm/3 hours)	Fertile group	Infertile group
0– 5	0	76
6–19	4	55
20–29	5	60
30–39	18	36
≥40	24	56

values of *L*, and the other semen qualities much lower *L* values.

The distribution of the samples with regard to sperm penetration extent for the fertile and infertile groups appears in Table VI. The difference of the distribution was significant ($X^2 = 27.7$, df $= 2$, $p < 0.01$). In Table VII the semen samples were distributed according to semen properties in relation to normal or low sperm penetration using 20 mm 3 hours as the limiting value. For all semen properties there was a higher incidence of samples with pathological quality and low penetration in the infertile group than in the fertile group. There was a higher incidence of samples with normal quality and high penetration in the fertile group than in the infertile group.

When the semen samples were grouped according to semen property findings (Table VIII) the mean sperm penetration values showed a clear difference between the fertile and infertile groups. For the fertile group the penetration was good even for samples of poor quality. For the infertile the mean penetration was clearly lower in samples of poor quality than in the normal ones.

Table VII. Distribution of the semen samples in the fertile and infertile groups according to sperm penetration and semen property findings

Semen property	Fertile group		Infertile group	
	<20 mm	>20 mm	<20 mm	>20 mm
Volume (ml)				
<2	1	3	20	16
≥2	3	44	110	136
Density (mill./ml)				
<20	0	0	31	3
20–59	2	0	15	10
≥60	2	47	86	138
Living ( )				
<70		8	90	73
≥70	2	39	51	87
Motile ( )				
<50	3	9	100	65
≥50	1	38	31	87
Motility degree				
0 1 2	3	1	87	27
3 4	1	46	44	125
Abnormal ( )				
50	2	7	91	56
<50		40	40	99
Fructose (mg)				
<100	0	10	10	11
≥100	4	37	121	141
Acid phosphatases (I.E.)				
<20 000	0	8	47	33
≥20 000	4	39	84	119

Table VIII. Mean sperm penetration values for the fertile and infertile groups distributed according to semen property findings

Semen property	Fertile group No.	Fertile group Mean penetration (mm)	Infertile group No.	Infertile group Mean penetration (mm)
Volume (ml)				
<2	4	33.0	36	17.4
≥2	47	34.5	247	19.9
Density (mill./ml)				
<60	2	28.5	59	10.6
≥60	49	34.6	[illegible]	22.4
Living ( )				
<70	10	30.1	163	16.3
≥70	41	35.3	120	23.9
Motile ( )				
<50	12	29.3	165	14.4
≥50	39	36.0	118	[illegible]
Abnormal spermatozoa ( )				
≥50	9	30.0	147	14.0
<50	42	35.3	136	25.6
Motility degree				
0, 1 or 2	4	20.5	114	10.0
3 or 4	47	35.6	169	26.1
Fructose (mg)				
<100	10	31.7	21	1.6
≥100	41	35.7	252	19.6
Acid phosphatase (I.E.)				
<20 000	8	38.1	80	14.3
≥20 000	43	33.7	203	21.7

DISCUSSION

Analysis of a semen sample is usually the main basis for assessing the male partner's role in an

infertile marriage. There is a great deal of variation in semen characteristics among repeated specimens from the same man (10). It is of great importance to include in the semen analysis all tests which can give information on fertility.

MacLeod & Gold (14) found that there was a closer relationship between the qualitative motility and the chance of conception than for any other of the semen properties. Sälo-Seidl (18) concluded that the qualitative motility of spermatozoa was much more important for assessing fertility than was the quantitative motility. Motility is usually subjectively judged, and cannot be exact. Qualitative and quantitative motility are closely correlated in the present material ($r = 0.71$) and both showed good correlation to sperm penetration. The discriminant function coefficient was higher for qualitative motility than for quantitative motility, but both values are lower than for sperm penetration. There are surely factors influencing penetration and not motility and perhaps vice versa.

Sperm penetration studies reported in the literature have been performed with different technics and direct comparisons cannot be made. The use of different test media and time spans has given different penetration extents. Botella-Llusiá (4) found that normal fertile spermatozoa penetrated about 27.4 mm into Ringer-fructose solution in one-half hour but only 7.1 mm in pure Ringer solution. He found a penetration of 20.9 mm in the same time period into cervical mucus. In the present series the mean sperm penetration in 3 hours was 34.4 mm for semen samples from the fertile group.

Investigating a group of 135 infertile couples Kremer (13) found a significantly better sperm penetration from semen samples with density 60 millions/ml, percentage of motile 40, motility degree > 3 (using a scale from 0 to 5). In regard to morphology he found not so obvious differences but significantly better penetration related to samples with $> 60\,\%$ normal headforms. Bergman (2) found that morphologically normal spermatozoa penetrated to a greater extent than abnormal, tested by postcoital analyses. In the present study there was found a definitely lower penetration for semen samples of subnormal quality.

Correlation coefficients between penetration and semen quality have not been reported in the literature nor do multiple correlation coefficients.

In the present material a relationship was found between penetration and cellular properties of the semen. Less obvious was the influence of volume, fructose and acid phosphatase in seminal plasma. The last mentioned 3 qualities were the only ones where no significant difference was found between the fertile and infertile groups. More tests of the function of the accessory genital glands are needed in order to explain the influence of these glands on the fertility of man. Compounds such as prostaglandins have been much studied, but the role they play in sperm transport is not clear (8).

The discriminant function analysis shows clearly that penetration has the highest discriminating power for the 2 groups. Motility degree and percentage of motile spermatozoa had lower discriminant function coefficients. This should indicate that the penetration test is the most reliable for assessing male fertility.

Semen of good quality almost invariably showed sperm penetration exceeding 70 mm 3 hours and the penetration was never less than 6 mm 3 hours. This is in good agreement with the results of Fjällbrant (9) investigating a group of men with sperm antibodies in serum. A classification with 20 mm 3 hours as a lower limit of normality can be proposed. The lower limit for fertility seems to be 6 mm 3 hours.

The results of the present investigation make apparent the interrelation between penetration and fertility. The variance of penetration due to regression of the semen qualities was only 43 %. The discriminant function analysis gave the highest L value for penetration and the distribution according to semen quality and penetration makes it obvious that penetration gives the best information about fertility. This should indicate that penetration is a valuable supplement to semen analysis giving information about fertility not obtained from other semen properties. The penetration test was evaluated earlier and found to be reliable with small variations for different ejaculates from the same man in spite of variation of the other semen properties (20). In this work only linear penetration was measured. The quantitative penetration, motility degree and duration of motility in cervical mucus of the penetrating spermatozoa might give further useful information.

Table VI *Distribution of the semen samples in the fertile and infertile groups according to sperm penetration extent*

Sperm penetration (mm/3 hours)	Fertile group	Infertile group
0–5	0	76
6–19	4	55
20–29	5	60
30–39	18	36
>40	4	56

values of *L*, and the other semen qualities much lower *L* values.

The distribution of the samples with regard to sperm penetration extent for the fertile and infertile groups appears in Table VI. The difference of the distribution was significant ($\chi^2 = 27.7$, df = 2, $p < 0.01$). In Table VII the semen samples were distributed according to semen properties in relation to normal or low sperm penetration using 20 mm 3 hours as the limiting value. For all semen properties there was a higher incidence of samples with pathological quality and low penetration in the infertile group than in the fertile group. There was a higher incidence of samples with normal quality and high penetration in the fertile group than in the infertile group.

Table VII *Distribution of the s men samples in the fertile and infertile groups according to sperm penetration and semen property findings*

Semen property	Fertile group		Infertile group	
	< 20 mm	> 20 mm	< 20 mm	> 20 mm
Volume (ml)				
<2	1	3	20	16
>2	3	44	110	136
Density (mill./ml)				
< 20	0	0	31	3
20–59	2	0	15	10
> 60		47	86	138
Living ( )				
< 70	2	8	90	73
> 70	2	39	51	87
Motile ( )				
< 50	3	9	100	65
> 50	1	38	31	87
Motility degree				
0 1 2	3	1	87	27
3 4	1	46	44	125
Abnormal ( )				
> 50	2	7	91	56
< 50		40	40	99
Fructose (mg)				
< 100	0	10	10	11
> 100	4	37	121	141
Acid phosphatases (I.E.)				
< 20 000	0	8	47	33
> 20 000	4	39	84	119

Table VIII *Mean sperm penetration values for the fertile and infertile groups distributed according to semen property findings*

Semen property	Fertile group		Infertile group	
	No.	Mean penetration (mm)	No.	Mean penetration (mm)
Volume (ml)				
<2	4	33.0	36	17.4
>2	47	34.5	47	19.9
Density (mill./ml)				
< 60	2	28.5	59	10.6
> 60	49	34.6	[illegible]4	[illegible]2.4
Living ( )				
< 70	10	30.1	163	16.3
> 70	41	35.3	120	[illegible]3.9
Motile ( )				
< 50	12	29.3	165	14.4
> 50	39	36.0	118	6.8
Abnormal spermatozoa ( )				
> 50	9	30.0	147	14.0
< 50	42	35.3	136	25.6
Motility degree				
0, 1 or	4	20.5	114	10.0
3 or 4	47	35.6	169	26.1
Fructose (mg)				
< 100	10	31.7	21	1.6
> 100	41	35.7	[illegible]52	19.6
Acid phosphatase (I.E.)				
< 20 000	8	38.1	80	14.3
> 20 000	43	33.7	203	21.7

When the semen samples were grouped according to semen property findings (Table VIII) the mean sperm penetration values showed a clear difference between the fertile and infertile groups. For the fertile group the penetration was good even for samples of poor quality. For the infertile the mean penetration was clearly lower in samples of poor quality than in the normal ones.

DISCUSSION

Analysis of a semen sample is usually the main basis for assessing the male partner's role in an

infertile marriage. There is a great deal of variation in semen characteristics among repeated specimens from the same man (10). It is of great importance to include in the semen analysis all factors which can give information on fertility.

MacLeod & Gold (14) found that there was a closer relationship between the qualitative motility and the chance of conception than for any other of the semen properties. Sillo-Seidl (18) concluded that the qualitative motility of spermatozoa was much more important for assessing fertility than was the quantitative motility. Motility is usually subjectively judged, and cannot be exact. Qualitative and quantitative motility are closely correlated in the present material ($r = 0.71$) and both showed good correlation to sperm penetration. The discriminant function coefficient was higher for qualitative motility than for quantitative motility but both values are lower than for sperm penetration. There are surely factors influencing penetration and not motility and perhaps vice versa.

Sperm penetration studies reported in the literature have been performed with different technics and direct comparisons cannot be made. The use of different test media and time spans has given different penetration extents. Botella Llusia (4) found that normal fertile spermatozoa penetrated about 27.4 mm into Ringer-fructose solution in one-half hour but only 7.1 mm in pure Ringer solution. He found a penetration of 20.9 mm in the same time period into cervical mucus. In the present series the mean sperm penetration in 3 hours was 34.4 mm for semen samples from the fertile group.

Investigating a group of 135 infertile couples Kremer (13) found significantly better sperm penetration from semen samples with density >60 millions/ml, percentage of motile >40, motility degree >3 (using a scale from 0 to 5). In regard to morphology he found not so obvious differences but significantly better penetration related to samples with >60% normal headforms. Bergman (2) found that morphologically normal spermatozoa penetrated to a greater extent than abnormal, tested by postcoital analysis. In the present study there was found definitely lower penetration for semen samples of subnormal quality.

Correlation coefficients between penetration and semen quality have not been reported in the literature nor do multiple correlation coefficients.

In the present material a relationship was found between penetration and cellular properties of the semen. Less obvious was the influence of volume, fructose and acid phosphatase in seminal plasma. The last mentioned 3 qualities were the only ones where no significant difference was found between the fertile and infertile groups. More tests of the function of the accessory genital glands are needed in order to explain the influence of these glands on the fertility of man. Compounds such as prostaglandins have been much studied but the role they play in sperm transport is not clear (8).

The discriminant function analysis shows clearly that penetration has the highest discriminating power for the 2 groups. Motility degree and percentage of motile spermatozoa had lower discriminant function coefficients. This should indicate that the penetration test is the most reliable for assessing male fertility.

Semen of good quality almost invariably showed sperm penetration exceeding 20 mm 3 hours and the penetration was never less than 6 mm 3 hours. This is in good agreement with the results of Fjällbrant (9) investigating a group of men with sperm antibodies in serum. A classification with 20 mm 3 hours as lower limit of normality can be proposed. The lower limit for fertility seems to be 6 mm 3 hours.

The results of the present investigation make apparent the interrelation between penetration and fertility. The variance of penetration due to regression of the semen qualities was only 43%. The discriminant function analysis gave the highest *L*-value for penetration, and the distribution according to semen quality and penetration makes it obvious that penetration gives the best information about fertility. This should indicate that penetration is a valuable supplement to semen analysis giving information about fertility not obtained from other semen properties. The penetration test was evaluated earlier and found to be reliable, with small variations for different ejaculates from the same man in spite of variation of the other semen properties (20). In this work only linear penetration was measured. The quantitative penetration, motility degree and duration of motility in cervical mucus of the penetrating spermatozoa might give further useful information.

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AMENORRHOEA FOLLOWING ORAL CONTRACEPTION

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… investigation of 31 patients who developed …rrhoea following the use of combined oral contra…ves for … period of 3 to 84 months revealed that … had … variable degree of oligomenorrhoea prior to …tment. It … therefore concluded that an antecedent …nal dysfunction is … relative contra-indication to …tment with combined oestrogen-gestagen preparations, … that another form of contraception should be recom…ded in these cases.

In 5 of the 31 patients the amenorrhoea … as associated … galactorrhoea. None of these patients had any signs … hypothalamic or hypophyseal tumour and no ex…nation can be given for this phenomenon.

Eighteen patients … ere observed … ithout any subsequent …onal treatment, 9 patients … ere treated … ith clomi…hene alone, 1 patient … ith clomiphene-HCG, 4 patients … ith HMG-HCG, and in 1 patient … bilateral ovarian re…ction … as performed. These forms of treatment resulted … ovulation in 16 patients and pregnancy in 5 patients.

On the basis of both the present and previous investiga…tions the following lines of treatment are recommended: In patients with no sterility problem the management should be expectant, … hereas hormonal treatment should be initiated in patients … ith infertility. Clomiphene either alone or combined … ith HCG in the treatment of choice in patients … ith … normal oestrogen level, but in patients with … low oestrogen level treatment … ith human gonado…trophins … preferred.

Since 1966 several cases of amenorrhoea following treatment with oral contraceptives ha e been reported (1 3 4 5 6 7 8, 9 10, 13 14 15 16, 17 18, 19 20, 21 23) and … causal relationship between the administration of these preparations and the subsequent amenorrhoea has been sug gested but not proved. The incidence of amenor rhoea after discontinuing therapy with oral contraceptives is unknown, but appears to be relatively small within the total population using these drugs. To the individual patient, however it re presents … significant complication and is a source of concern, since the problem often occurs … t time when the patient has a desire for pregnancy.

The purposes of the present article are 1) to define the women especially at risk of developing amenorrhoea following oral contraception and) to establish certain lines of the treatment of these patients.

MATERIAL AND METHODS

The series consists of 31 patients, … ho … ere referred to our department during the period 1969 to 1971 because of secondary amenorrhoea occurring after cessation of treat ment … ith oral contraceptives. None of these patients had received any hormonal treatment prior to the ad mission. Table I show … the most important clinical data for the 31 patients. Ages ranged from 18 to 34 years, … ith an average of … 4 years. T enty-two patients … ere nulli-gravida, 7 were primi-gravida, and 2 … ere secunda-gravida. Ages at menarche varied from 10 to 16 years, the average being 14 years. The menstrual pattern prior to the treatment … ith oral contraceptives, … as show … in Table I. 14 patients had regular menstruations, whereas 4 patients had slight oligomenorrhoea … ith cycles of 30–60 days duration, and 13 patients had severe oligomenor rhoea. None of the patients had previously had secondary amenorrhoea, … no menstruation for … period of 6 months or more.

Nine different combined oestrogen-gestagen prepara tions … ere used by the patients included in the present series. The treatment period varied from 3 to 84 months … ith an … verage of 25 months, and all patients had received the treatment primarily for contraceptive pur poses. The duration of the amenorrhoea prior to the first examination varied from 6 to 33 months, … ith an average of 12 months. 1 … 5 of the 31 patients (nos 1 8, 12, 23 31) the amenorrhoea … as associated … ith … slight galactor rhoea.

All patients were admitted to hospital for endocrine evaluation, including determinations of the urinary excre tion of 17-ketosteroids (22), 17-ketogenic steroids (12), total oestrogens (2), and total gonadotrophins (11), and the concentration of protein bound iodine (PBI) in serum. I … addition, an endometrial biopsy was performed in all patients and, in order to exclude … hypophyseal tumor,

Table I. *Clinical data for 31 patients with amenorrhoea following oral contraception*

Patient no.	Age (years)	No. of pregnancies	Menarche (years)	Previous cycle (days)	Preparation	Duration of therapy (months)	Duration of sec. amenorrhoea (months)	Galactorrhea
1	20	0	13	28–38	Lyndiol Ovulen	41	6	+
2	20	0	15	28–36	Nonovul	4	12	–
3	23	0	14	30–180	Lyndiol	36	8	–
4	24	0	16	30–180	Delpregnin	48	15	–
5	23	0	13	30–60	Plan	21	7	–
6	27	0	13	30–60	Anovlar	30	15	–
7	34	0	15	30–150	Enavid Gynovlin	84	6	–
8	34	1	10	30–60	Lyndiol	15	33	+
9	23	0	16	30–180	Gynovlar	5	9	–
10	18	0	12	30–90	Eugynon	6	9	–
11	24	0	14	30–180	Gestovex	30	12	–
12	23	0	14	30–180	Ovulen	3	8	+
13	22	0	13	28–30	Unknown	36	9	–
14	22	1	13	30–32	Ovulen	20	1	–
15	23	2	14	30	Delpregnin	24	11	–
16	26	0	13	30	Delpregnin	30	10	–
17	25	0	12	90–180	Gestovex	24	8	–
18	20	0	13	30–120	Delpregnin	24	6	–
19	21	0	13	90–180	Lyndiol	24	9	–
20	22	0	12	60–90	Delpregnin	14	7	–
21	24	0	12	30–180	Enavid Lyndiol	48	11	–
22	23	0	12	30–90	Lyndiol	18	13	–
23	29	1	12	28	Delpregnin	30	15	+
24	27	2	13	28–35	Ovulen	36	17	–
25	33	1	13	28–30	Nonovul	17	6	–
26	26	0	14	30	Lyndiol	27	16	–
27	25	1	14	28	Ovulen Eugynon	22	15	–
28	23	1	14	28	Delpregnin	11	7	–
29	26	0	12	30–60	Gestovex Ovulen	14	17	–
30	20	0	13	28	Gestovex Delpregnin	11	13	–
31	24	1	13	26–30	Anovlar	18	18	+

radiological examination of the sella turcica was carried out and the fields of vision determined. Finally an examination for X-chromatin was carried out in all cases.

FINDINGS

Table II shows the hormonal status of the 31 patients investigated. The values given in this table are means of 2 determinations. The excretion of both 17-ketosteroids (17 KS) and 17-ketogenic steroids (17 KGS) was within the normal range in all patients except for no. 28 in whom the excretions of both 17 KS and 17 KGS were moderately increased. This patient was overweight and slightly hirsute. The concentration of PBI in serum was within the normal range in all patients, and for this reason the results are not given in the table.

The excretion of total oestrogens ranged from 2 to 33 μg/day which is significantly lower than the normal range of 20 to 80 μg/day in menstruating and ovulating women. The excretion of total gonadotrophins was determined biologically and expressed in mouse uterine units/day (MUU/day). The excretion of gonadotrophins was within the normal range in all patients except no. 22, in whom no gonadotrophin output was detectable (less than 3 MUU/day).

The next column in Table II indicates the development and phase of the endometrium obtained by biopsy. Nine out of the 31 patients showed an atrophic endometrium while the remaining

Table II. *Hormonal status and treatment of 31 patients with amenorrhoea following oral contraception*

Patient no	17-KS (mg/day)	17-KGS (mg/day)	Total oestrogens (µg/day)	Total gonadotrophins (MUU/day)	Endometrial biopsy	Problem of sterility	Treatment (months)	Result: Menstruation	Result: Ovulation	Result: Pregnancy
1	11.0	8.8	21	12	Prol. phase	No	Observation (2)	+	+	–
2	6.7	7.3	4	6	Atrophic	No	Observation (9)	–	–	–
3	14.0	11.0	23	19	Prol. phase	No	Observation (6)	–	–	–
4	7.8	8.2	16	32	Prol. phase	No	Observation (4)	–	–	–
5	8.4	7.6	33	10	Prol. phase	No	Observation (3)	–	–	–
6	10.8	10.0	2	7	Atrophic	No	Observation (3)	–	–	–
7	8.1	5.6	14	15	Prol. phase	No	Observation (4)	+	–	–
8	10.3	13.2	9	10	Atrophic	No	Observation (6)	–	–	–
9	13.5	10.8	8	6	Atrophic	No	Observation (3)	–	–	–
10	8.1	8.2	6	5	Atrophic	No	Observation (11)	+	–	–
11	8.5	7.8	33	9	Prol. phase	No	Observation (3)	–	–	–
12	5.7	4.9	7	8	Prol. phase	No	Observation		–	–
13	9.5	5.9	5	5	Atrophic	No	Observation (8)	–		–
14	10.2	10.4	—	15	Prol. phase	No	Observation (3)	+		–
15	10.8	9.8	—	23	Prol. phase	No	Observation (1)	+		–
16	6.2	7.1	8	9	Prol. phase	Yes	Observation (3)	–		–
17	9.8	9.4	26	23	Prol. phase	Yes	Observation (2)	–		
18	13.4	13.4	22	17	Prol. phase	Yes	Observation (3)	+		+
19	6.3	8.1	7	8	Atrophic	Yes	Clomiphene (1)	–	–	–
20	14.0	11.7	10	33	Prol. phase	Yes	Clomiphene (1)			–
							Observation (4)			–
21	8.9	4.8	30	4	Prol. phase	Yes	Clomiphene (3)		+	–
22	13.0	7.6	15	<3	Prol. phase	Yes	Clomiphene (1)			–
							HMG–HCG (3)			–
23	13.5	6.5	6	23	Atrophic	Yes	Clomiphene (3)		–	–
							HMG–HCG (2)		+	+
24	11.8	9.4	22	21	Prol. phase	Yes	Clomiphene (1)	–		+
25	6.4	9.6	18	4	Prol. phase	Yes	Clomiphene (1)	+		–
26	13.7	12.4	9	5	Prol. phase	Yes	Clomiphene (3)			–
27	10.3	8.3	4	16	Atrophic	Yes	Clomiphene (1)	+	–	–
28	17.0	14.2	17	12	Prol. phase	Yes	Clomiphene–HCG (1)	–	+	+
29	7.8	6.3	11	10	Prol. phase	Yes	HMG–HCG (2)	+		–
30	8.6	6.5	7	8	Atrophic	Yes	HMG–HCG (2)		+	–
31	13.9	11.0	21	33	Prol. phase	Yes	Ovarian reaction	+	+	–
							Observation (2)			

22 patients showed endometrium in the proliferative phase. There is a good correlation between the excretion of total oestrogens and the development of the endometrium.

In all cases the radiological examination revealed a normal sella turcica, and the fields of vision were normal. All patients were X-chromatin positive.

TREATMENT AND RESULTS

Table II shows that 16 out of the 31 patients had involuntary sterility whereas 15 patients were worried only because of the amenorrhoea. During the whole period it has been our conviction that stimulation therapy with either clomiphene or human gonadotrophins should only be used in patients with sterility and in most cases clomiphene alone or combined with human chorionic gonadotrophin (HCG) was our first choice of treatment. In all other cases it has been our policy to reassure the patients, wait for spontaneous cure without any treatment, and offer stimulation therapy whenever a desire for pregnancy should occur.

Eighteen patients (nos. 1 to 18) were observed without any hormonal treatment for a period varying from 1 to 11 months. In all cases the occurrence of menstruation, ovulation and pregnancy was recorded. Only patients with typical biphasic basal body temperature curves and those who became pregnant were recorded as ovulating.

Table I *Clinical data for 31 patients with amenorrhoea following oral contraception*

Patient no.	Age (years)	No. of pregnancies	Menarche (years)	Previous cycle (days)	Preparation	Duration of therapy (months)	Duration of sec. amenorrhoea (months)	Galactorrhea
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5	23	0	13	30–60	Plan	21	7	–
6	27	0	13	30–60	Anovlar	30	15	–
7	34	0	15	30–150	Enavid Gynovin	84	6	–
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9	23	0	16	30–180	Gynovlar	5	9	–
10	18	0	12	30–90	Eugynon	6	9	–
11	24	0	14	30–180	Gestovex	30	12	–
12	23	0	14	30–180	Ovulen	3	8	+
13	22	0	13	28–30	Unknown	36	9	–
14	[illegible]	1	13	[illegible]	Ovulen	20	12	–
15	23	2	14	30	Delpregnin	24	11	–
16	26	0	13	30	Delpregnin	30	10	–
17	25	0	12	90–180	Gestovex	24	8	–
18	20	0	13	30–120	Delpregnin	24	6	–
19	21	0	13	90–180	Lyndiol	24	9	–
20	22	0	12	60–90	Delpregnin	14	7	–
21	24	0	12	30–180	Enavid Lyndiol	48	11	–
22	23	0	12	30–90	Lyndiol	18	13	–
23	29	1	12	28	Delpregnin	30	15	+
24	27	2	13	28–35	Ovulen	36	17	–
25	33	1	13	28–30	Nonovul	17	6	–
26	26	0	14	30	Lyndiol	27	16	–
27	25	1	14	28	Ovulen Eugynon	22	15	–
28	23	1	14	28	Delpregnin	11	7	–
29	26	0	12	30–60	Gestovex Ovulen	14	17	–
30	20	0	13	28	Gestovex Delpregnin	11	13	–
31	24	1	13	26–30	Anovlar	18	18	+

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7 out of the 18 patients had a spontaneous menstruation within the observation period, 5 patients showed ovulation and 2 of these became pregnant (Table II)

Nine patients (nos. 19 to 27) were treated with clomiphene alone and 1 patient (no 28) with clomiphene HCG for 1 to 3 cycles. Six of the 10 patients menstruated following the treatment, 6 patients showed ovulation and 2 of these patients became pregnant.

Four patients (nos. 22, 23 29 and 30) were treated with human menopausal gonadotrophin (HMG) followed by HCG for a period of 2 to 3 cycles All 4 patients menstruated following the first treatment cycle and they all ovulated in every treatment cycle but only 1 became pregnant.

One patient (no 31) had a bilateral ovarian resection performed because of moderately enlarged polycystic ovaries. This patient was observed for 2 cycles following the operation. She had spontaneous menstruation and ovulation but she did not become pregnant.

DISCUSSION

There is no adequate data on the prevalence of secondary amenorrhoea in the normal population nor on its incidence in women who have stopped treatment with oral contraceptives. However Rice-Wray et al. (17) found that 2.8% of their patients failed to menstruate for periods of 3 to 12 months after cessation of oral contraception. It is worthy of note that in a few cases amenorrhoea may occur after treatment for only 3–4 months (Table I).

Reviewing the literature I have found 184 cases of amenorrhoea after discontinuance of oral contraception in which there is sufficient information about the menstrual pattern prior to treatment. It is remarkable that 63 (34%) of these patients had previous oligomenorrhoea or amenorrhoea, whereas 121 (66%) previously menstruated regularly The frequency of previous oligomenorrhoea among these patients was confirmed by the present study in which 55% of the patients had greater or lesser degrees of oligomenorrhoea prior to oral contraception. This finding is important, and it is my opinion that a patient with oligomenorrhoea, who requests oral contraception should be informed of this potential complication and another kind of contraception recommended. On the other hand, it must be admitted that many of these patients with amenorrhoea have a history of regular menstruation prior to treatment, and therefore no individual is without the potential risk of this complication.

Several cases of amenorrhoea associated with galactorrhea following oral contraception have been reported (5 7 20) In the present series 5 of the 31 patients had slight galactorrhea. None showed any signs of a hypothalamic or hypophyseal tumor The occurrence of galactorrhea is difficult to explain It has been suggested that these patients may produce a hormone which causes lactation (presumably prolactin) or which interferes with the production of one of the normal pituitary hormones, the presence of which inhibits lactation.

On the basis of previous studies and my own experience I recommend the following lines of direction for treatment of patients with amenorrhoea after oral contraception. If the amenorrhoea has lasted for more than 6 months a physical examination and endocrinologic evaluation should be made in order to exclude other causes of amenorrhoea. In all patients not complaining of sterility the management should be expectant, as the rate of spontaneous resolution is acceptable In the present study without any of 18 patients having treatment during the first few months of observation 5 were cured and this finding is in good agreement with previous studies (16 19) In patients with infertility the treatment has to be more active The treatment of choice depends especially on the oestrogen level as it has been shown both in the present and previous investigations (7 8 21) that patients with a normal excretion of oestrogens and a well developed endometrium respond well to treatment with clomiphene either alone or combined with HCG whereas patients with a low oestrogen excretion and an atrophic endometrium do not ovulate on this treatment. In the latter cases, therefore treatment with HMG-HCG is the best treatment, and nearly all patients will respond to this therapy In contrast, the excretion of gonadotrophins is of less importance and serves only to exclude those very few patients who have an increased output of gonadotrophins, and in whom any treatment will be unsuccessful.

Acta Obstet Gynec Scand 51 347–350, 1972

THE EFFECT OF TRANEXAMIC ACID (AMCA) ON POSTOPERATIVE BLEEDING AFTER CONIZATION

Göran Rybo and Hans Westerberg

From the Department of Obstetrics and Gynaecology (Head: Professor Sam Brody), University of Göteborg, Göteborg 5, Sweden

Abstract. A double blind randomized trial was made to ascertain the effect of tranexamic acid (AMCA) (Cyklokapron®) on the postoperative blood loss after conization. The case material consisted of 50 women referred to the clinic because of dysplasia or non-invasive cancer of the cervix. Five patients were excluded for various reasons. The treatment started in the evening of the day of operation and was continued for another 12 days, the dose being three tablets every 8 hours, corresponding to 4.5 g of tranexamic acid daily when the active drug was given. During the first 7 postoperative days, when the patients were in hospital, the blood losses were determined quantitatively. Prophylactic treatment with tranexamic acid reduced the postoperative blood loss as compared with the placebo group, the blood losses being 23 ± 3.2 ml and 79 ± 20.4 ml respectively. Sudden profuse bleeding postoperatively, requiring remedial measures, occurred in 7 patients, all in the placebo group. With the exception of 1 patient in the placebo group, who complained of nausea, no side effects were recorded.

The conization technique for non-invasive cancer of the cervix may be complicated by postoperative bleeding. There are two types of such bleeding. One consists of a continuous, successively decreasing loss of blood during the postoperative period, whereas the other is a sudden, heavy blood loss, usually occurring within the first 2 weeks following the operation. The latter complication often requires such measures as transfusions or further surgery. Some authors have reported good results in reducing bleeding complications after conization when epsilon-aminocaproic acid (EACA) is used as a prophylactic agent (2, 3). However, in these investigations, the blood loss was not determined quantitatively but estimated by the fall in the haemoglobin concentration in venous blood.

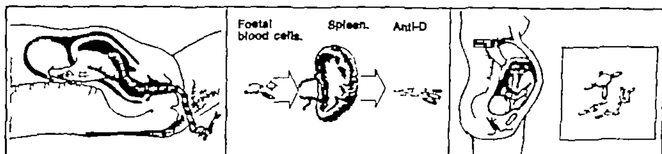
The mode of action of EACA involves the suppression of the activation of plasminogen to plasmin. Endometrial tissue is known to contain a relatively high concentration of plasminogen activators (6) and the menstrual blood loss can be reduced by administering drugs with an inhibitory effect on plasminogen activation (4, 5). Furthermore, plasminogen activators are present in the cervical tissue (3). The bleeding following operation on the cervix may be due to the action of these activators on the haemostatic mechanism.

The introduction of a new antifibrinolytic agent, tranexamic acid (AMCA, Cyklokapron®, AB KABI, Stockholm, Sweden), involves lower dosages and produces fewer side effects than treatment with EACA, although the effect on fibrinolytic bleeding is reported to be the same with both preparations (4, 5). In view of this, a study of the effect of tranexamic acid on blood loss after conization of the cervix seemed to be of value. Consequently, a clinical trial was performed to compare the effect of tranexamic acid with that of a placebo, employing a double-blind technique with randomization.

MATERIAL AND METHODS

The series consisted of 50 non-selected women (aged 23–62) referred to the Department of Obstetrics and Gynaecology because of dysplasia or non-invasive cancer of the cervix. The diagnosis had previously been established by means of cytology, curettage and/or biopsies from the portio. The patients were operated on by different surgeons according to a standard scheme used in the clinic. The portio was stained by means of the Schiller technique, and the descending branches of the uterine artery were ligated. Thereafter a cone, with its base on the portio and its apex pointing towards the internal cervical os, was excised. The base included all the

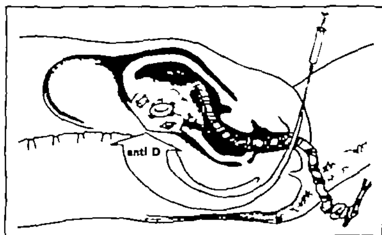
Prevention of Rh-immunization



Schematic illustration of how a small amount of blood crosses over from the foetus to the mother during parturition.

Foetal Rh-positive blood cells elicit the formation of antibodies in the Rh-negative mother

Antibodies pass over from the Rh-immunized mother to the foetus.



Prophylactic administration of Gammaglobulin anti-D to the mother within 72 hrs. after delivery in order to prevent Rh-immunization

Gammaglobulin anti-D Kabi

without mercury Storage refrigerator Shelf-life 3 years.
Pack 250µg ampoules comb package



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Table I. *Blood loss in connection with conization in 22 women treated with tranexamic acid*

Patient no.	Blood loss (ml)	Day after operation when sudden, profuse bleeding occurred	Remarks
1	20		Used oral contraceptives
2	17		
3	44		
4	4		
6	10		
8	3		
12	24		
15	41		
17	39		
19	11		
22	14		
28	21		
29	26		
30	19		
33	24		
36	24		
37	63		
38	9		Used oral contraceptives
42	27		
45	10		
48	13		
49	46		
Mean value	23 ± 3.2		

Schiller-negative parts. Stitches were applied to bleeding vessels until the bleeding stopped. The wound cavity was not covered by so-called Sturmdorf sutures, in order to avoid the risk of enclosing dysplastic tissue in cervical crypts, which might reduce the reliability of the post operative cytological follow-up examination. After operation a perforated glass tube was usually inserted in the cervical canal and a pack in the vagina. The pack was removed on the following day but the glass tube was generally left for 4 to 5 days.

Tranexamic acid therapy was started on the evening following operation, three tablets being administered. Alternatively three tablets of a placebo were given. The tablets were similar in shape and taste. Each tablet of the active substance contained 0.5 g tranexamic acid. Therapy was continued for 12 more days in a dosage of 3 tablets every 8 hours. Thus, each patient treated with the active substance received a total of 4.5 g of tranexamic acid per day which amounts to 55.5 g for the whole period of treatment. The period in hospital ranged from 7 to 10 days. Administration of drug or placebo was by random selection.

No other drug was given during the investigation. However 2 patients in the tranexamic acid group (nos. 1 and 38) and 3 in the placebo group (nos 5, 1 and 27) were on oral contraceptives before the operation, and this medication was not discontinued.

For 7 days after operation, during which time the patients were in hospital, all sanitary towels, tampons etc., were collected, and the blood loss was determined quantitatively by the method described by Hallberg & Nilsson (1). These determinations were performed in order to evaluate the postoperative blood loss. Furthermore, a record was made of any sudden more profuse bleeding which required such measures as transfusion, infusion of plasma expanders (Macrodex®), injection of antifibrinolytic agents or resuturing for control of blood loss. For practical reasons, it was not possible to measure such blood loss.

The blood loss during operation was not determined, since differences in surgical technique might cause considerable variations in the blood loss. Any side effects which occurred during treatment were recorded.

Five patients were excluded before the code was broken. Three patients (nos. 13 and 25 in the tranexamic acid group, and no. 24 in the placebo group) were excluded because during the course of the operation they had been given by mistake a vasopressin analogue (Octapressin®) by infiltration into the cervix, 1 patient (no. 9 in the placebo group) was excluded because she had been given EACA intravenously immediately after the operation in order to stop bleeding induced by surgery and with the fifth patient (no. 20 in the tranexamic acid group), there was some uncertainty as to whether she had been treated as scheduled and, consequently she was also excluded. Thus the tranexamic acid group consisted of 22, and the placebo group of 23 patients.

RESULTS

Tables I and II show the continuous blood losses during the postoperative week in women treated with tranexamic acid and the placebo respectively. The mean values were 23 ± 3.2 ml and 79 ± 20.4 ml. The difference between the mean values is statistically significant when tested by Student's t test ($p < 0.05$).

The results were also calculated for both groups after excluding the women taking contraceptives. The mean value of the blood loss in subjects who were given tranexamic acid was then 24 ± 3.5 ml and in those given the placebo 87 ± 2.9 ml. Apparently these exclusions did not influence the results. The difference between the mean values is statistically significant ($p < 0.05$).

Sudden profuse bleeding occurred postoperatively in 7 patients, all of whom were in the placebo group. It is of interest to note that none of the patients who received tranexamic acid had this bleeding complication. Such bleeding occurred from 5 to 10 days after operation (Table II). In 3 of the patients (nos. 11, 13 and 31) resuturing was necessary and they were also treated

Table II. *Blood loss in connection with conization in 23 women treated with placebo*

Patient no.	Blood loss (ml) during 7 postoperative days	Day after operation when sudden, profuse bleeding occurred	Remarks
5	30		Used oral contraceptives
7	285		
10	13		
11	372	6	Sutured. Treatment with blood and EACA.
13	63	7	Sutured. Treatment with blood and EACA
14	13		
16	18		
18	263		
21	7		Used oral contraceptives
26	40	7	Treatment with EACA in Macrodex infusion
27	37		Used oral contraceptives
31	124	5	Sutured. Treatment with blood and EACA.
32	127	6	Treatment with EACA and Macrodex
34	38		
35	30		
39	62		
40	63	10	Treatment with tamponade and EACA.
41	90		
43	96		
44	31		
46	22		
47	3		
50	25	8	Readmission to hospital. Treatment with EACA and Macrodex
Mean value	79 ± 20.4		

with blood transfusion and EACA. In 3 cases (nos. 26, 32 and 50) bleeding was controlled by means of EACA and Macrodex® and in 1 case (no. 40) treatment consisted in tamponade and EACA.

With the exception of 1 patient in the placebo group, who complained of nausea, no side effects were recorded.

DISCUSSION

Conization of the portio-cervix has become increasingly common as a result of cytological screening of the population for carcinoma of the cervix. Though cone-biopsy is a relatively simple procedure, there is always a risk of postoperative bleeding. In some cases bleeding necessitates blood transfusions or infusion of plasma expanders or even surgery. Consequently, in many clinics a relatively long period in hospital following conization is customary.

The results of the present investigation show that prophylactic administration of an antifibrinolytic drug, such as tranexamic acid, reduces the "small" continuous postoperative blood loss. It is important, however, to recognize that also the frequency of sudden, profuse bleeding, which sometimes occurs postoperatively, may be effectively reduced. This type of complication often requires repeated surgery or transfusion. Administration of an antifibrinolytic agent not only seems to be very effective in preventing such complications, but may also contribute towards reducing the duration of stay in hospital, to the advantage of both the patient and the economy of the hospital.

The present study also shows that the postoperative bleeding complications after conization are minimized when tranexamic acid is administered prophylactically. Virtually the same results were obtained by Jensen (2) and Kullander (3) using another antifibrinolytic preparation—EACA. However, in their investigations the postoperative blood losses were not determined objectively. The effect of the blood loss on the haemoglobin concentration was used as para-

meter and postoperative bleeding which necessitated the application of special measures was recorded. It is reasonable to assume that the effect of antifibrinolytic drugs is due to an inhibition of the action of the plasminogen activators present in the cervical tissue.

Oral contraceptives influence many processes in the human body The effect of contraceptive pills on the haemostatic mechanism is still obscure but the possibility of such influence cannot be rejected. Some of the patients in this study were using oral contraceptives before they were admitted for conization. This medication was not discontinued during or after operation. The inclusion of these patients did not, however affect the results.

Differences in surgical technique may cause variation in the postoperative blood loss. Even if a standardized operative technique was used, such a source of error could not be excluded since different surgeons performed the operations. However the fact that the double-blind method was applied in this trial and that differences in the surgical procedure must have occurred in both the placebo group and the tranexamic acid group minimizes the risk that such an error could affect the results.

In view of the results of this study it may reasonably be concluded that tranexamic acid administered prophylactically after conization, reduces postoperative bleeding complications.

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UNILATERAL PREGNANCY IN THE RABBIT

A Comparison of the Spontaneous Uterine Activity and Response to Neurohypophysial Hormones of the Nonpregnant and Pregnant Horns

Lennart Petersohn

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Abstract. In 24 conscious unilaterally pregnant rabbits the spontaneous and oxytocin-induced uterine activity was recorded by either one or two sponge-tipped catheters placed in each horn. In the pregnant horn the catheters were placed between the fetal membranes and the uterine wall. The recordings were repeated daily during the last days of pregnancy for 4 to 8 days post partum, and thereafter at longer intervals for up to several weeks. The pregnant horn was consistently more active and more sensitive to oxytocin than the sterile horn up to about 1 week after delivery but the difference disappeared. Intravenous vasopressin injections up to 0.05 IU did not increase the activity in either horn.

In pregnancy the myometrium is influenced by a series of factors, some of them acting systemically e g circulating hormones and the autonomic nervous system, others having local influence, e g myometrial stretching due to increasing uterine volume and the local effect of placental hormones (3). These two groups of factors can be studied separately in animals with double uteri, where one uterine horn has been made sterile. Isolated observations have indicated interesting deviations in the behaviour of the non-pregnant and pregnant horns in rabbits (11 and 7). The purpose of the present investigation was to study the problem of general versus local factors in a series of unilateral pregnancies in rabbits, effected by ligation of one of the Fallopian tubes.

MATERIAL AND METHODS

Twenty-four rabbits of the Swedish Land race were used, mean weight 3.8 kg. The mean duration of pregnancy in these rabbits is 31 days. The animals were anaesthetized with intravenous pentobarbitone sodium (Mebumalnatrium, ACO Sweden). Laparotomy was performed by incision in the right flank under sterile conditions. The right Fallopian tube was ligated with fine silk thread. The rabbits were mated about 3 weeks later and pregnancy, which was unilateral, was confirmed by palpation about 1 week before expected delivery. Further laparotomy was performed, using midline incision about 2 to 4 days before expected delivery (in one case 9 days before expected delivery). Sterile vinyl catheters (Becton and Dickinson, ID/OD 0.5 mm/0.9 mm), filled with isotonic sodium chloride solution and tipped with a small sponge were inserted in both horns through small incisions in the uterine wall. The sponge was about 5 mm long, cylinder shaped and with a diameter scarcely greater than that of the catheter (2). Usually one catheter was placed in each horn, but in 4 cases two catheters were placed in each uterine horn, one at the cervical end and one at the tubal end. In the pregnant horns the catheter tips were placed between the fetal membranes and the uterine wall. The uterine incisions were closed with sutures, which also secured the catheters. The abdominal wall was sutured and the catheters were led subcutaneously to an incision in the skin of the neck where they were secured with thread (11). At the same time, a polyethylene catheter (Portex, England, ID/OD 1.14 mm/1.57 mm) was inserted by a cut down in the right external jugular vein. This catheter was also led subcutaneously to the neck incision.

Recordings of the uterine activity were usually begun the day after the second operation and repeated daily until parturition occurred. Daily recordings were made for 4 to 8 days post partum and thereafter at longer intervals for up to several weeks. During recordings the animals were conscious, but they were placed in a small box which restricted their movements. The intra-uterine pressure changes were measured by means of a pressure transducer (Elema-Schönander, EMT/34), an amplifier (Elema-Schönander, EMT/31) and a three-channel potentiometer (Rika-Denki). The transducer was placed at the uterine level, which thus represented the zero level.

In addition to recording the spontaneous uterine activity we investigated the response to synthetic oxytocin

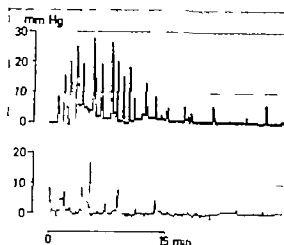
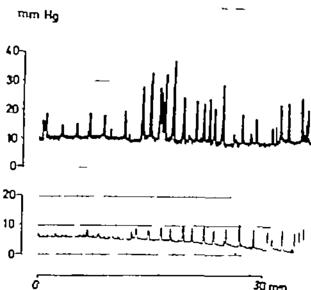
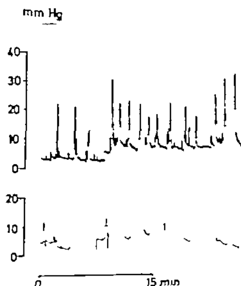


Fig. 1. Spontaneous uterine activity during parturition (delivery of the last pup in the litter) in a unilaterally pregnant rabbit, 31 days post coitum. Upper trace: pregnant uterine horn. Lower trace: sterile horn.





(Syntocinon, Sandoz) and, in 5 cases, to 8-lysine vasopressin (Postacton, Ferring).

The drugs were injected through the intravenous catheter in 1.0 ml isotonic saline solution and washed in with a further 1.0 ml saline. Oxytocin, in doses varying between 0.005 IU and 0.1 IU was given during intrauterine pressure recording. There was one exception, a rabbit in which intra-uterine pressure recording was commenced during the course of a spontaneous delivery. Vasopressin, in doses varying between 0.01 IU and 0.05 IU was given after oxytocin to the first 5 animals. This treatment was subsequently discontinued since it caused death of the fetuses without clearly influencing uterine activity.

RESULTS

Spontaneous activity

Before parturition the pressure changes were in general of small amplitude and low frequency with some variation from individual to individual. In all cases, spontaneous uterine activity was greater in the pregnant than in the sterile horn regarding, both frequency and amplitude. Recordings of intra-uterine pressure on succeeding

mm Hg 40 30 20 10 0 30 20 10 0 0 15 min

Fig. 2. (a) Spontaneous uterine activity recorded days post partum from a rabbit which had been unilaterally pregnant. Upper trace: left uterine horn, which had contained 4 fetuses. Lower trace: sterile right horn. (*b*) Spontaneous uterine activity recorded 9 days post partum in the same rabbit. The tracings as in (a). (c) Spontaneous activity recorded from another rabbit, 13 days post partum. Upper trace: left uterine horn which had contained 4 fetuses. Lower trace: sterile right horn.

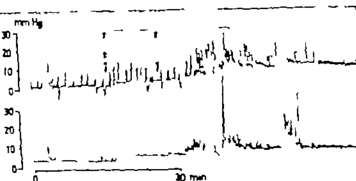


Fig. 3. Spontaneous and oxytocin induced activity in unilaterally pregnant rabbit, 31 days p.c. Upper trace: pregnant horn. Lower trace: sterile horn. In the period of frequent contractions 3 pups were delivered, weighing 52.2 g, 48.8 g and 28.0 g.

days revealed no clear change in the pattern of spontaneous activity preceding the induction of parturition with oxytocin. The pattern of uterine activity during a spontaneous delivery (Fig. 1) did not seem to differ from that during induced labour described below. After parturition, the uterus was very active. The frequency and especially the amplitude of the pressure changes varied considerably from rabbit to rabbit but was always greater in the delivered horn than in the control horn (Fig. 2 *a*). However, this difference had largely disappeared after about 1 week (Fig. 2 *b*, *c*).

In those rabbits which had two catheters in each horn, no difference in activity pattern could be observed at the two ends. The problem of contraction waves could not be judged by this set-up with only two catheters in a long horn.

Response to oxytocin

Parturition was most often induced by oxytocin on the 30th or 31st day p.c. An example of oxytocin induction of labour is shown in Fig. 3. The spontaneous and oxytocin induced activity is much lower in the sterile than in the pregnant horn. One delivery was obtained on the day after catheter insertion, 27 days p.c. and two occurred on the 29th day. There was also one rabbit which did not deliver at all, discussed separately below.

Oxytocin in doses of 0.005 IU or 0.01 IU

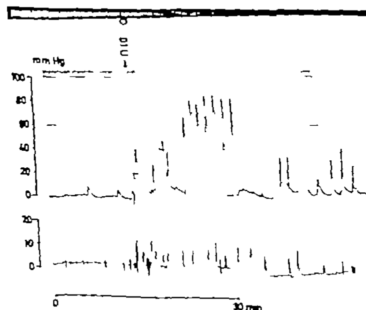


Fig. 4. Spontaneous and oxytocin induced uterine activity one day post partum in a rabbit which had been unilaterally pregnant. Upper trace: left uterine horn, which had contained 3 fetuses. Lower trace: sterile right uterine horn. Note the large difference in the pressure scales between the upper and lower traces.

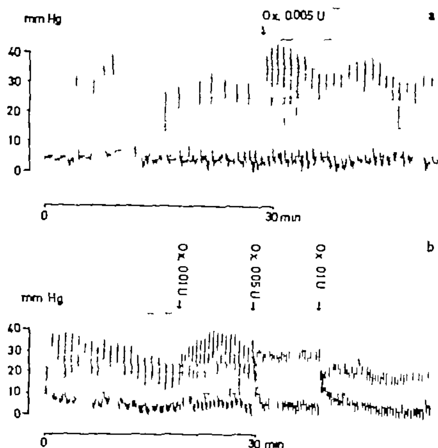


Fig. 5. Spontaneous and oxytocin induced uterine activity in the pregnant horn of a unilaterally pregnant rabbit. (a) 29 days p.c. (b) 33 days p.c.

usually gave no response up to the 29th day p.c. On the 30th day these doses induced a clear response in 5 rabbits. On these doses 2 of them delivered the same day, the other 3 on the fol lowing day. Larger doses of oxytocin, 0.05 IU and 0.1 IU, induced a myometrial response in one rabbit on the 27th day p.c. (i.e. 3 days before delivery) and in 2 rabbits they induced labour on the 29th day p.c.

During the first week post partum the uterus responded to oxytocin, 0.005 IU and 0.01 IU, by an increase in both frequency and amplitude of the pressure peaks (Fig. 4).

The response to oxytocin with regard both to frequency and to amplitude was always greater in the pregnant horn than in the sterile horn. Similarly, during the first week post partum the delivered horn gave a more pronounced response than the sterile one.

Before delivery the response to oxytocin was obtained about 1 minute after the injection whether or not the dose induced parturition. During the post partum period on the other hand, the response was always instantaneous.

One rabbit deviated clearly from all the others. We inserted the catheters on the 22nd day p.c. and began to record intra-uterine pressure on the following day. Spontaneous, frequent and strong contractions were obtained from the start, the pregnant horn being by far the most active. On the 29th day a very strong and almost instantaneous response to the injection of 0.005 IU oxytocin (Fig. 5 *a*) and an even stronger response to 0.01 IU was recorded from the pregnant horn. The same response was obtained on the following days and became even more pronounced, the amplitude of the contractions recorded being greater than in any other rabbit before parturition (Fig. 5 *b*). From the 31st day p.c. even the non-pregnant horn showed a mild response with an increase in amplitude and frequency. No delivery occurred however and 1 week after the expected date of parturition the rabbit was sacrificed and examined. In the pregnant horn we found three very macerated fetuses.

Response to vasopressin

In the five rabbits which received vasopressin in doses of up to 0.05 IU we could not with cer tainty establish any effect upon uterine activity

These rabbits eventually delivered on oxytocin on the expected day of parturition but all the young were born dead. In rabbits which received only oxytocin, most or all of the litter three to six young, were alive at birth.

In four experiments, we ligated the left fallopian tube at the time of catheter insertion. The recordings obtained from these rabbits did not deviate in any way from the others.

DISCUSSION

In the pregnant uterine horn spontaneous uterine activity during pregnancy parturition and puerperium was similar to that previously recorded from unanaesthetized rabbits (11 and 7) Broad agreement with the results of other workers was found in several other respects. Oxytocin sensitivity increased at the end of pregnancy (16 and 11) and labour was induced by oxytocin injection at or near term (8 13 and 15). A positive myometrial response to synthetic vasopressin has previously been described only for the puerperal rabbit (12). We observed little or no change in the intra-uterine pressure pattern when vasopressin was injected into pregnant or puerperal rabbits. In this respect the pregnant rabbit clearly differs from the pregnant woman, whose uterus is rather sensitive to vasopressin (10). On the other hand, vasopressin administration led to death of the fetuses (14 1 and 4) presumably due to the reduction in maternal placental blood flow induced by vasopressin (6 and 5).

In all cases—before term, at term, during delivery and after delivery—the pregnant horn was more active and more sensitive to oxytocin than the sterile horn. This difference must be due to local factors as both horns are under the same general influence, e.g. by circulating ovarian hormones. The experiments do not indicate the nature of the local factors. One possible factor is the local hormonal effect of the placenta (see extensive review ref 9) another the obvious anatomical difference between the two horns. The fact that the difference in spontaneous and oxytocin induced activity between the two horns persists about one week after delivery may suggest that the anatomical difference is of major importance, because the hormonal factors should have equalized a few days after delivery

… an active and oxytocin sensitive myometrium already on the 23rd day p.c. later delivered very macerated fetuses. It is possible that the fetal death occurred before the first recording, in which case the pregnancy was abnormal.

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ULTRASONIC PELVIMETRY

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Abstract. In recent years high accuracy in measuring the biparietal diameter by ultrasonography has been achieved. As this is only one parameter in forecasting cephalo-pelvic disproportion, a study was made to determine whether the true conjugate can be measured by ultrasound with approximately the same reliability as the biparietal diameter. First, one-dimensional ultrasound studies were compared with X-ray measurements. Later, equipment was used which allows simultaneous display on two oscilloscopes of the B-scan for orientation and the A-mode trace for exact measurement. To exclude disproportion in the mid-pelvis a vaginal rectal probe was constructed. In longitudinal scans, information about the form of the birth canal is obtainable. Transverse scans performed at the level of the symphysis provide information on the pelvic entrance. To evaluate ultrasonic pelvimetry the outcome of labour in 246 cases was analysed. The study demonstrates that ultrasonic pelvimetry can be an invaluable supplementary aid to X-ray diagnosis of cephalo-pelvic-disproportion especially in cases of breech presentation. Because it is often possible to diagnose cephalo-pelvic disproportion conclusively by using ultrasonic pelvimetry, X-ray examination can be restricted to borderline cases. In this manner it is possible therefore to reduce radiation dosage.

The prognosis and outcome of labour is dependent upon many factors. The two most important parameters in labour are the shape and width of the pelvis and the largest non-deformable part of the fetal head. The relation between the true conjugate of the pelvis and the fetal biparietal diameter is of greater importance than the absolute measurements. In normal cases the true conjugate which is the line connecting the promontory to the nearest part of the posterior wall of the symphysis measures approximately 11 cm, less half a centimetre due to the muscle layer.

Different methods can be used to measure this distance. For example, an estimate can be made by subtracting 9 cm from the external conjugate as measured by callipers, or by vaginal examination.

Guthmann's X-ray technique provides reliable results in measuring the true conjugate. Simultaneous measurement of the fetal biparietal diameter is only possible if the head presents; in breech presentation, measurement of the biparietal diameter becomes extremely unreliable due to technical factors.

Since ultrasonic cephalometry can be performed with high accuracy we tried to use sonar examination techniques for pelvic mensuration also.

Murooka's paper (6) provided the incentive for us to develop an ultrasonic method for this purpose.

Our first observations, on patients suffering from cervical cancer, were later on confirmed by Pystynen (4), Rosenow et al. (7), and Loch (8).

METHOD

"Combison" ultrasonic equipment, manufactured by Kretztechnik was used in this study. This equipment allows simultaneous display on two oscilloscopes of the B-scan for orientation and the A-mode trace for exact measurement. Initially we used only the A-mode technique.

The transducer was mounted on the symphysis. Because of an air-cushion created by the pubic hair a large amount of contact medium is required. High gain is necessary to penetrate the symphysis and to receive an echo from the promontory. Because of this high gain it is impossible to separate the echo corresponding to the posterior wall of the symphysis from the very broad emitting signal. Therefore the promontory echo is first marked and then the gain is reduced until it becomes possible to define the echo corresponding to the posterior wall of the symphysis. The distance between these two echoes should correspond to the true conjugate.

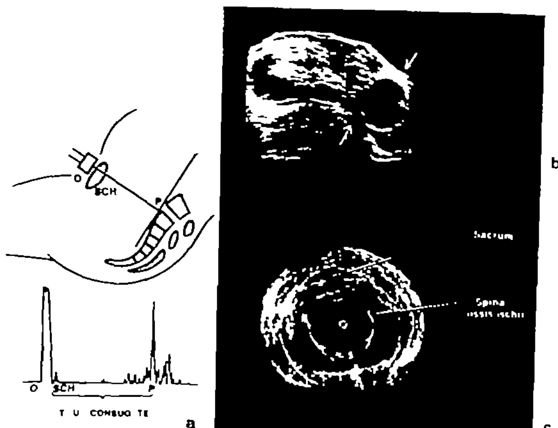


Fig 1 Methods for ultrasonic pelvimetry (*a*) left side of the picture measuring the true conjugate by the A-mode technique *O* = emitting signal, *Sch* = symphysis, *P* = promontory (*b*) outlining the promontory by longitudinal scan from the xyphoid sternum to the symphysis. The promontory and the symphysis are marked by arrows. The bright base-line of the B-scope is directed in such a way that the measurement can be taken simultaneously on the continuous A-scope. ( ) measuring the interspinous distance by using an intra-vaginal or intra-rectal rodlike transducer.

It has become easier to locate the promontory following the introduction of ultrasonic tomography We now perform multiple longitudinal scans in the midline until both the spine and the promontory can be identified. Because of the bright base-line of the B-scope the path of the emanated ultrasonic waves is always visible By directing the beam in such a way that the echoes corresponding to the promontory and the symphysis can be identified simultaneously the exact measurement on the A-scope is possible by means of electronic callipers.

Structural obstetrical problems are not always located in the pelvic entrance but sometimes in the mid-pelvis as well. We therefore developed the vaginal transducer for the measurement of the interspinous distance. For this purpose the rodlike probe is inserted into the rectum Parallel radial scans are provided at intervals of 1 cm. The ischial spines are usually situated approximately 6–7 cm above the anus.

In longitudinal scans information about the form of the birth canal also is obtainable.

Recently we have employed a further scanning plane to outline the pelvic entrance as well.

The scan is performed with the patient supine. The scanner head is positioned at the upper margin of the symphysis and inclined in such a manner that the transducer points towards the promontory In such a scan the pelvic entrance the femural heads, and the trochanters can be identified At the same time it is possible to estimate the distance of the fetal head from the promontory and the symphysis.

RESULTS

With the use of these methods from 1966 to 1971 334 patients were referred for ultrasonic pelvimetry (Table I) From 46 patients the obstetrical report was available for comparison with the prognosis given by ultrasonic pelvimetry and the outcome of labour

These patients were grouped according to the measurement of the true conjugate. The outcome of labour was divided into the following groups: spontaneous delivery, vacuum extraction or forceps delivery, manual aid and cesarean section.



Fig. 2. Different forms of the birth canal. (*a*) longitudinal scan with normal birth canal, showing clearly the promontory and the sacrum, fetus in cephalic position. (*b*) demonstrates very long and flat birth canal. (*c*) demonstrates an examination in contracted pelvis with the head overlapping the symphysis.



Fig. 3. The upper scan is longitudinal scan outlining the fetus but as ell as the promontory and the sacrum. *P* = promontory, *S* = symphysis and sacrum, *R* = trunk, *K* head, *H* skin. Below: Transverse scan at the level of the upper margin of the symphysis, the scanner head tilted in such manner that the transducer face faces towards the promontory. The scan reveals the femoral heads and the sacrum as well as the fetal head.

The table well illustrates that the estimation of the true conjugate by calliper measurements of the external conjugate is an unreliable method.

As the results from 1966 to 1968 are already published elsewhere, we restricted our analysis to the results from 1969 to 1971 (Table II). During this time w were able to evaluate ou prognosis in 190 patients. In 31 patients, that is 16.31% cesarean sections were necessary (Table III).

Since the absolute size of the true conjugate and the fetal biparietal diameter was not of major interest to us, we developed comparison system for the relative difference in size of the pelvis and

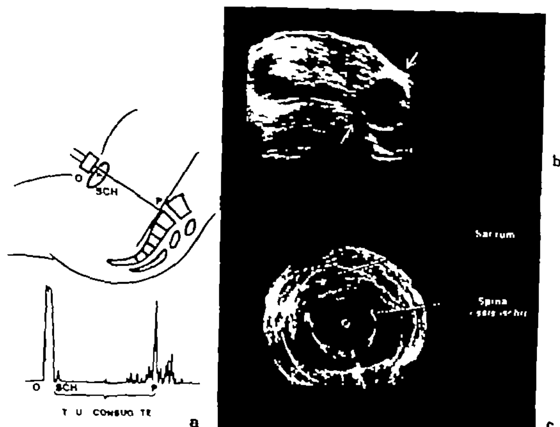


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same as a week earlier The resident on duty doubted the reliability of the ultrasonic measurements and requested a pelvic X-ray The results differed from each other by only 1 mm. Although the cervix was dilated to 6 cm, the membranes had not yet ruptured and the fetal head was still floating in an undescended position. At this point the resident began to doubt the results of both examinations. Being convinced of the reliability of the measurements performed, the membranes were ruptured, the head descended ithin a short time and the mother delivered ithout further complication.

In this group there were only two serious discrepancies where interpretation of the available data was at fault.

Prognosis and outcome of breech presentation as followed by our staff very closely (Table VII). The only case with an absolute indication for cesarean section because of cephalo-pelvic disproportion was correctly diagnosed ant partum. In the other 5 cases a cesarean was performed for other reasons

Table IV

Difference between b p d. and true conjugate (mm)	Cases	Cesarean for p d	Cesarean for other reasons	Total
0–15	32	12	—	12
16–20	43	2	2	4
21–25	46	2	5	7
26–30	50	5	3	8
30	19	—	—	—
Total	190	21 (11 05)	10 (5.26)	31

Table V

True conjugate	Cesarean section Difference in mm between b p d. and true conjugate					
	0–10	11 15	16–20	21 25	26–30	Total
6 5–8 9	1/					
9 0–10 0	6/					1
10 1–11 0	3/					6
11 1–12 0		1/	2/1	/1	1/	9
12 1–13 0		1/	/1	2/4	3/	11
					1/3	4
Total	10/	2/	2/2	2/5	5/3	31

*3 T12004

Table VI

Cephalo-pelvic disproportions
21 cases

Diagnosed by Sonar examination	14	*Not diagnosed*	7
Difference less than 10 mm	10	Functional	1
Vertexposition	2	Kyphoscoliosis	1
Flat canal pelvis	2	Normal size but c. in history	2
		Hip operation	1
		Failures	2

Table VII

True conjugate	Breech presentation Difference between b p d. and true conjugate						
	0–10	11 15	16–20	21 25	26–30	Total	Cesarean
6.5–8 9							
9 0–10 0	1					1	1
10 1 11.0			1	1		2	2
11 1 12.0			3	7	5	15	1
12.1–13 0				2	3	5	1
Total	1		4	10	8	23	5

DISCUSSION

Ultrasonic pelvimetry seems to be valuable aid in the diagnosis of cephalo-pelvic disproportion. This was reinforced by Pystynen s findings where he compared his results in pregnant women with X-ray measurements. Ultrasonic pelvimetry was shown to be as reliable as X-ray examination in the cases reported by Loch

Although we are of the opinion that ultrasonic pel imetry is not always as accurate as X-ray pelvimetry we were readily able to forecast the outcome in two cases with long and flat birth canals, using only ultrasonic tomography

The diagnosis of cephalo-pelvic disproportion in vertex presentation can be made readily by this technique. In the one-dimensional examination we are never able to obtain a midline echo and the measured distance is always larger than x pected for the period of gestation. I ultrasonic tomography we can obtain very typical echograms. In longitudinal scans especially in cases of occipito posterior position, only the occipital part of the fetal skull is clearly outlined. The f ce beneath the abdominal wall is of irregular and ill-defined shape.

Table I

Examined patients	334
Obstetrical reports available	246
Not available	88

the fetal head. This was particularly important in cases of breech presentation and cesarean section. The problem with breech presentation is that one must be aware of a cephalo-pelvic disproportion prior to labour in order to avoid serious com plication (Table IV)

The figures to the left of the slashmark in-dicate the sections performed because of cephalo-pelvic disproportion. The figures to the right (Table V) indicate cesareans performed for other reasons. The highest incidence of sections for cephalo-pelvic disproportion is in the group where the difference between the two parameters is less than 10 mm although. Even if the difference is larger many sections are still indicated for cephalo-pelvic disproportion

In comparing our prognosis with the outcome of labour in these cases by studying the ob-stetrical reports, we found that we had correctly diagnosed 14 out of 21 cases of disproportion prior to delivery (Table VI)

The group of 7 patients, where a discrepancy between our prognosis and the outcome of labour existed was of special interest.

The first case was one of functional cephalo-pelvic disproportion. The true conjugate was of normal size the difference between the biparietal diameter and the true conjugate was 13 mm. The baby weighed 4 400 g and was 53 cm in length. Two years later the same patient had a spontane ous vaginal delivery of a baby weighing 3 200 g and 50 cm in length.

Two other patients with a history of cesarean section for cephalo-pelvic disproportion performed at another clinic also were of interest. Although the true conjugate measured by ultrasound was normal the operation was performed a few hours after onset of labour with a cervical dilatation of only 3 cm. We believe that in these cases the decision for repeated cesarean section was strongly influenced by the history

An interesting case developed in the following manner A primigravida on whom we had per formed ultrasonic pelvimetry for nondescent of the head was admitted. The situation was the

Table II

				Delivery by				
1966–71 Group	Cases n	Extern. conjug.	True conjug.	manual aid	cumm forceps	cesar sect.	spontan.	Total
I	1	16.0	6.5			1		1
II	16	17–21	9 0–10.0		2	10	4	16
III	62	17 21	10.1 11.0	3	4	13	42	62
IV	125	17– 1 5	11 1–12.0	18	7	14	86	125
V	4[illegible]	18–22	12.1–13.0	5	4	4	29	42
Total	46			26	17	42	161	246

Table III

				Delivery by				
1969–71 Group	Cases n	Extern. conjug.	True conjug.	manual aid	vacuum forceps	cesar sect.	sponta	Total
I	1	16.0	6.5			1		1
II	8	17 21	9.0–10.0		1	6	1	8
III	43	17–21	10.1 11 0		4	9	30	43
IV	103	17 21.5	11 1 1[illegible]0	14	4	11	74	103
V	35	18–22	1[illegible]1 13 0	4	3	4	4	35
Total	190			18	12	31	129	190

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THE EFFECT OF OESTRADIOL ON THE ARTERIAL DIAMETER IN MICE, AS STUDIED BY ANGIOGRAPHY

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Abstract. Ten spayed female mice received a subcutaneous injection of 1 µg of oestradiol-benzoate. Whole body angiography was performed on the oestrogen-treated and untreated controls. The diameters of the arteries supplying the head, neck, fore limbs, pelvis and posterior limbs were measured with an optical micrometer. The arteries of the head, neck and fore limbs in the oestrogen-treated group were smaller in diameter, while the diameter of the internal iliac artery (hypogastric trunk) was larger when compared with controls. The results are discussed in relation to recent findings in humans.

Treatment with oestrogen of ovariectomized mice was followed by a significant decrease and disintegration of the mast cells in the genital tract while the mast cells of a peripheral organ (the skin) were unaffected. The clotting time of peripheral blood was unaltered (11, 12 and 5).

It is only recently that a valid cause and effect relation has been established between the oestrogen component of oral contraceptives and several factors involved in the development of circulatory disorders (3, 4, 6, 7, 8 and 10). The reported side effects of the oestrogen component of oral contraceptives made it important to investigate the influence of oestrogen on the arterial diameter of the main arteries under experimental conditions by means of angiography.

MATERIAL AND METHODS

Twenty white, healthy inbred female mice of fertile age with a mean weight of 24 g were ovariectomized under ether anesthesia. During the second week after spaying, daily vaginal smears were taken to confirm non-cyclic activity. The mice were divided into two groups of 10. Two weeks after castration, the experimental group (OE) received a subcutaneous injection of 1 µg of oestradiol-mono-benzoate in 0.5 ml sesame oil. The second group served as controls (C) and received 0.5 ml of sesame oil subcutaneously (Table 1).

On the 5th day after treatment the animals were rendered unconscious by a sharp blow to the head. A immediate intracardiac injection of a suspension of 50 Micropaque (Damancy & Co) and 50 ml of distilled water was made via the left ventricle. The suspension was infused at a constant pressure of 100 mg Hg for 10 min and needles with the same internal diameter (0.4 mm) were used throughout the experiment. Rectal temperature and the temperature of the Micropaque suspension were maintained at 37°C.

Whole body radiographs in a dorso-ventral view were taken on Agfa non-screen film, using a custom-built X-ray unit. The exposure data were 56 kV 50 mA and 0.8 sec with a film-focus distance of 175 cm. The X-ray films were developed for 4 min at 20°C in a standard commercial developer.

The X-ray films were magnified 10 times on photographic paper. On this photograph the fronto-occipital and the biparietal diameters as well as nose-rump length were measured. The diameters of the following arteries were measured bilaterally 5 mm beyond their origin: internal carotid, common carotid, subclavian, brachial, common iliac, internal iliac and femoral. The arteries were measured using an ocular micrometer (enlargement 8) enabling measurement of 0.1 mm. Because of the long film-focus distance and the small and similar size of experimental and control animals the enlargement factor was negligible.

Double blind measurements were made by two different individuals. The standard error of the measurement was calculated to be 2%. The data were analysed statistically and differences were considered significant when $p < 0.01$. A post-mortem examination was performed to confirm complete ovariectomy.

RESULTS

One mouse was omitted from the oestradiol-treated group because of inadequate ovariectomy

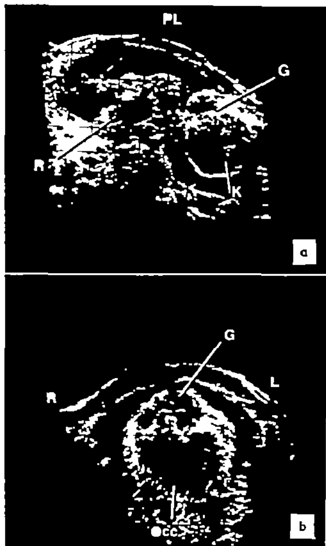


Fig 4 ( ) A longitudinal scan in a patient with the fetus in occipito posterio position. Typical is the clear-cut occiput and the irregular face *Pl* = placenta, *R* = trunk, *G* = face *K* = head, *P* = promontory *E* = extremities, *F* = fluid. (*b*) A transverse scan just above the symphysis, outlining the occiput and the anteriorly located face with the orbit.

Additional transverse scans in such cases allow us to confirm the diagnosis. The broad occiput is located posteriorly and the smaller forehead anteriorly Sometimes even the fetal orbits or the zygomatic area can be recognized

Ultrasonic pelvimetry is of great advantage in all cases of breech presentation. Using X ray methods one is able to measure exactly the true conjugate and even the interspinous distance. As it is impossible to estimate the distance of the fetal head from the film plane, no correction can be made for the enlarged fetal skull on the film, caused by the diverging X ray beam. To eliminate this inaccuracy Goldberg et al. (3) took a polaroid film of the fetal head at its largest circumference and superimposed the pictures after enlarging the headfilm to the same scale as the X ray of the pelvis. In this manner he was able to forecast cephalo-pelvic disproportion in his cases.

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Table II. *Influence of oestradiol on the arterial diameter in ovariectomized mice*

Group	Internal carotid art.	Comm. carotid art.	Subclav art	Brachial art.	Comm. iliac art.	1st iliac art.	Femoral art.
C	2.65±0.05	4.23±0.08	3.92±0.08	2.91±0.03	3.24±0.07	1.13±0.12	2.35±0.09
OE	1.96±0.05	3.97±0.06	2.49±0.08	2.16±0.06	3.03±0.08	1.85±0.09	2.43±0.07
C>OE (t-value)	7.36	6.15	3.49	4.50*	0.84	2.26	0.32

Mean values of the diameter of the arteries and standard error of the mean in mm (enlargement 10) and their corresponding t-values.
** p 0.001 p 0.01 C control group. OE = oestradiol treated group.

meter between oestrogen and control groups occurs using physiological dosages of oestrogen can not be settled at present. The findings of Gabrielsen & Greitz, 1970 suggest that even normally the diameters of the cerebral arteries are influenced by oestrogen.

It would seem worth while to utilize available clinical cerebral angiographic material to throw further light on this problem. One would like to raise the following questions:

1. Do oral contraceptives taken by females of fertile age influence the width of their cerebral arteries.
2. Does oestrogen administered to women with premenopausal symptoms affect their cerebral vascular tone?

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Table I. *Treatment schedule of mice*

c = castration weeks before treatment, – = no treatment, s = subcutaneous injectio of 0.5 sesame oil oe = subcutaneous injection of 1 μg oestradiol-mono-benzoate in 0.5 ml sesame oil + = angiography

Group	No of animals	Pretreatment	Treatment on day 1	2	3	4	5
C	10	c		–	–	–	+
OE	10	c	oe	–	–	–	+

while 2 mice from the control group were omitted because the needle was erroneously introduced into the right ventricle. There were no differences in skull diameters or body length between the oestrogen and control group. The diameters of the arteries supplying the head, the neck and the fore limbs of the oestradiol-treated group were smaller than the corresponding vessels in the control group. There were no differences between the diameters of the arteries supplying the pelvis and the posterior limbs in the oestradiol treated and control groups. However the internal iliac arteries (hypogastric trunk) were wider in the oestradiol-treated group than in the control group (Fig. 1 and Table II)

DISCUSSION

In several mammalian species lack of oestrogen causes atrophy of the genital organs. It is therefore to be expected that the arteries supplying the genital tract should increase in diameter under the influence of this hormone This has also been shown to be true in the human (1)

Our findings of larger arterial diameters at the cephalic end of the body in spayed female mice are in conformity with a recent angiographic study in the human (2) After correction for skull size the cerebral arteries in females were found to be narrower than in males. There was also a tendency toward increased size of the cerebral arteries with advancing age.

Although some information is available concerning the effect of oral contraceptives on amino-acid metabolism and mental depression (9) it is not possible to explain our findings on the basis of the report mentioned.

Among possible explanations the following seems most likely Oestrogen may mediate a stimulation of 5-hydroxytryptamine sensitive adrenergic alpha receptors in the CNS thereby increasing the vascular tone. Ageing, castration, or lack of oestrogen might on the other hand enhance certain enzyme systems such as monoamino-oxidase thereby making less 5-hydroxytryptamine available with a reversed effect on the vascular tone.

The amount of oestrogen administered in the present paper is in the pharmacological range but this has also been true for oral contraceptives. Whether or not the difference in arterial dia-

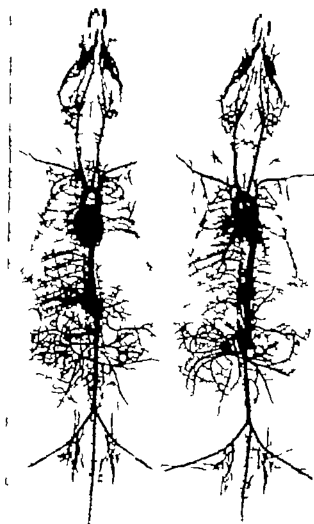


Fig 1 Whole body-angiograms, dorso-ventral view f oestrogen-treated mouse (left) and control (right). The diameters of the internal carotid, common ca otid, subclavian and the brachial arteries are smalle In the oestrogen-treated mouse than in the control The reverse relation f the internal iliac arteries is also demonstrated.

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EXTERNAL CARDIAC MASSAGE AS A METHOD OF TREATMENT OF NEONATAL ASPHYXIA

A Cineangiographic Study in the Piglet

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Abstract Eight piglets were delivered at term by abdominal hysterotomy They were prevented from breathing The umbilical vein and artery and the jugular vein were cannulated. Arterial blood pressure and ECG were recorded and, when heart activity had ceased, cineangiography was performed with injection of contrast medium into the umbilical vein, or with injection through the jugular vein catheter, advanced to the superior vena cava. When contrast medium was injected into the umbilical vein it passed through the right atrium to the left atrium and left ventricle When it was injected into the superior vena cava it was distributed to the right and left atrium and also to the right ventricle External cardiac massage forced contrast medium into the arterial system. Evidently contrast medium thereby reached the ascending aorta, the coronary arteries, and the brachiocephalic artery more readily following injection into the umbilical vein than into the superior vena cava. By the latter route, it could be distributed partly through the ductus arteriosus to the descending aorta. Infusion of oxygenated blood in the heart at standstill resulted in vigorous heart contractions

Oxygenated adult blood infused into the umbilical vein of the severely asphyxiated newborn clearly improves cardiac activity (3 4) It increases the heart rate and thereby the systolic blood pressure Since this effect was elicited even though the infusion corresponded to no more than 3–5 % of the total blood volume, it was assumed that the blood infused into the umbilical vein was following the fetal route, i.e., it was taking the shortcut through the ductus venosus and the foramen ovale to the left ventricle and the ascending aorta. The infused oxygenated blood would thereby reach the coronary arteries with minimal admixture The study to be reported in this paper was undertaken to test the validity of this assumption and to find out if in the neonate external cardiac massage can at all replace the pumping action of the heart when myocardial activity has ceased to exist. Severely asphyxiated newborn piglets were examined using cineangiography The contrast medium was injected into the umbilical vein and, for comparison, also into the superior vena cava.

MATERIAL AND METHODS

Laparotomy was performed on pregnant sow at term under phencyclidinhydrochloride (Sernylan, Parke Davis & Co) anesthesia Eight fully-developed piglets, weighing 0 95–1 50 kg, were delivered by hysterotomy The myometrium was incised at a site where a piglet head could be palpated through the uterine wall and the head was clearly visible in the amniotic sac, which bulged through the incision To prevent the piglet from breathing, a saline-filled rubber glove was slipped over its snout before the membranes were ruptured. After aeration of the lungs had thus been rendered impossible, the piglet was delivered. Polyethylene catheters (PE 160) were inserted into an artery and a vein of the umbilical cord. When their tips were at the umbilicus, they were fixed in this position by tying a cotton ribbon around the umbilical cord The catheter in the artery was used for transmitting blood pressure to a transducer and the vein catheter was used for injection of contrast medium For the latter purpose also, a similar catheter was inserted into the jugular vein and, when it was seen under fluoroscopy that its tip was in the superior vena cava close to the right atrium, the catheter was fixed in that position.

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EXTERNAL CARDIAC MASSAGE AS A METHOD OF TREATMENT OF NEONATAL ASPHYXIA

A Cineangiographic Study in the Piglet

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Abstract. Eight piglets were delivered at term by abdominal hysterotomy. They were prevented from breathing. The umbilical vein and artery and the jugular vein were catheterized. Arterial blood pressure and ECG were recorded and, when heart activity had ceased, cineangiography was performed with injection of contrast medium into the umbilical vein, or with injection through the jugular vein catheter advanced to the superior vena cava. When contrast medium was injected into the umbilical vein it passed through the right atrium to the left atrium and left ventricle. When it was injected into the superior vena cava it was distributed to the right and left atrium and also to the right ventricle. External cardiac massage forced contrast medium into the arterial system. Evidently contrast medium thereby reached the ascending aorta, the coronary arteries, and the brachiocephalic artery more readily following injection into the umbilical vein than into the superior vena cava. By the latter route, it would be distributed partly through the ductus arteriosus to the descending aorta. Infusion of oxygenated blood in the heart at standstill resulted in vigorous heart contractions.

Oxygenated adult blood infused into the umbilical vein of the severely asphyxiated newborn clearly improves cardiac activity (3, 4). It increases the heart rate and thereby the systolic blood pressure. Since this effect was elicited even though the infusion corresponded to no more than 3–5% of the total blood volume, it was assumed that the blood infused into the umbilical vein was following the fetal route, i.e., it was taking the shortcut through the ductus venosus and the foramen ovale to the left ventricle and the ascending aorta. The infused oxygenated blood would thereby reach the coronary arteries with minimal admixture. The study to be reported in this paper was undertaken to test the validity of this assumption and to find out if in the neonate external cardiac massage can at all replace the pumping action of the heart when myocardial activity has ceased to exist. Severely asphyxiated newborn piglets were examined using cineangiography. The contrast medium was injected into the umbilical vein and, for comparison, also into the superior vena cava.

MATERIAL AND METHODS

Laparotomy was performed on pregnant sows at term under phencyclidinhydrochloride (Sernylan, Parke Davis & Co.) anesthesia. Eight fully-developed piglets, weighing 0.95–1.50 kg, were delivered by hysterotomy. The myometrium was incised at a site where a piglet head could be palpated through the uterine wall and the head was clearly visible in the amniotic sac, which bulged through the incision. To prevent the piglet from breathing, a saline-filled rubber glove was slipped over its snout before the membranes were ruptured. After aeration of the lungs had thus been rendered impossible, the piglet was delivered. Polyethylene catheters (PE 160) were inserted into an artery and the vein of the umbilical cord. When their tips were at the umbilicus, they were fixed in this position by tying a cotton ribbon around the umbilical cord. The catheter in the artery was used for transmitting blood pressure to a transducer, and the vein catheter was used for injection of contrast medium. For the latter purpose also, a smaller catheter was inserted into the jugular vein and, when it was seen under fluoroscopy that its tip was in the superior vena cava close to the right atrium, the catheter was fixed in that position.

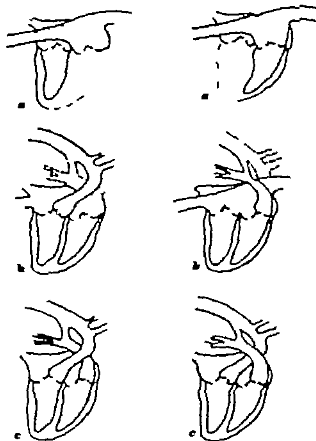


Fig 1 Fig 2

Fig 1 Distribution f contrast medium infused into the umbilical vein. Lateral view Schematic drawings from cinéradiograms. Shaded area outlines distribution of contrast medium. Dashed lines indicate borders of unfilled spaces. ( ) Filling of both atria and *left* ventricle before cardiac massage was initiated. (*b*) Filling of ascending aorta, cephalic arteries and right ventricle after the first cardiac massage movement. ( ) Propagation of contrast medium into pulmonary arteries and ductus arteriosus with continued cardiac massage.

Fig 2 Distribution of contrast medium infused into the superio vena cava. Symbols as in Fig. 1 ( ) Filling of both atria and *right* entricle before cardiac massage (*b*) Filling f pulmonary artery ductus arteriosus, descending aorta and left ventricle after initial cardiac massage mo ements. ( ) Propagation of contrast medium into ascending aorta and cephalic arteries with continued cardiac massage

Arterial blood pressure, as well as standard limb lead II of the ECG, were recorded continuously and, when these methods of examination indicated cessation of heart acti ity the radiographic contrast medium (meglumin-amido-trizoate, Angiografin, Schering Co.) was infused.

It was gi en *via the umbilical vein cathete* as a rapid injection of 4 ml (4 injections in 2 piglets) or as a continuous drip infusion lasting 15 min (1 experiment). One piglet, which had been given a bolus f 4 ml, was immediately given a further rapid injection, this time consisting of 2 ml contrast medium mixed with 8 ml heparinized maternal blood, which had been oxygenated as described by Enhörning et al., 1972 (4). This injection of contrast medium/blood mixture was repeated twice In 6 piglets, 4 ml of contrast medium was injected rapidly *via the jugular vein catheter* (8 injections).

The cinéradiographic studies were made before, during, and after infusion of contrast medium and external cardiac massage, using a Triplex optimatic 1023 (Elema-Schönander 3-phase 200 KVP) with image, intensifier, television monitor and an Arriflex 35 mm cinécamera. Twenty exposures per second were made on Eastman Plus-X (Reversal Film SO 273). With electronic contactors, the X ray exposures were synchronized with the film camera in a ciné pulse system. Exposure data ere 60 kV 80 mA, and exposure time 0.003 sec.

When after each infusion the distribution of contrast medium had been recorded on cinéfilm, external cardiac massage was started. While the camera was operating, 8 to 10 digital pressures were exerted, one per second, over the heart.

For each type of infusion the piglets were alternatively placed either on their left or right side

RESULTS

Contrast medium infused into umbilical vein

Regardless of whether the contrast medium was given by fast injection or by slow infusion, its propagation was as follows. Via the ductus venosus it reached the inferior vena cava from which it entered the right and left atrium and subsequently also the left ventricle There was slight retrograde filling of the vena hemiazygos and the abdominal parts of the vena cava. Even after the first digital pressure exerted during cardiac massage contrast medium became visible in the ascending aorta, the brachiocephalic artery the carotid arteries the left brachial artery and the right ventricle With continued cardiac massage filling of the pulmonary artery the ductus arteriosus and the descending aorta became evident (Fig. 1)

The piglet which, after a dose of 4 ml of contrast medium, was given oxygenated blood, responded as follows. The first bolus of contrast medium was distributed as just described With no cardiac massage the second injection consisting of 2 ml of contrast medium together with 8 ml of oxygenated blood was given When this mixture reached the heart, there was a series of 20 powerful heart contractions visible on the angiocardiographic film and registered on the ECG The very first ventricular contraction forced the contrast medium into the ascending aorta as

all as into the right ventricle. As a reflection of this left ventricular output, systolic blood pressure increased to 75 mmHg. With the subsequent ventricular contractions, the contrast medium was forced into the pulmonary arteries and the ductus arteriosus. This course of events was repeated in the two following runs.

Contrast medium infused into superior vena cava

The rapidly injected contrast medium promptly entered the right as well as the left atrium, but was then distributed backwards into the superior vena cava and the S-shaped proximal part of the vena hemiazygos, and into the inferior vena cava down to the hepatic branches. The contrast medium then continued to fill the superior vena cava to the level of the upper aperture of the thorax. In 5 of the 8 infusions the contrast medium was seen to enter the right ventricle. In the remaining 3 infusions only atrial filling was demonstrated.

With the initial cardiac massage contrast medium was forced into the pulmonary artery and through the ductus arteriosus to the descending aorta. The ascending aorta was not outlined. The following massage projected the contrast medium to the left ventricle and to the ascending aorta. The brachiocephalic artery, the carotid arteries and the left brachial artery also became outlined (Fig. 2).

DISCUSSION

In 1958 closed chest cardiac massage was already used in the clinical schedule for resuscitation (9). Today external cardiac massage combined with ventilation is a generally accepted treatment for severe neonatal asphyxia (2 and 8). Yet, in the newborn, the efficacy of this therapy has not been experimentally tested. In the severely asphyxiated adult dog it is possible with external cardiac massage, to raise pressure in the femoral artery and to maintain an adequate carotid blood flow (5). However the finding that cardiac massage can force blood to move forward is not necessarily valid in the neonate with persisting fetal shunts. In newborn infant, Moya et al. (7) were able to demonstrate that pressures in the right atrium and the aorta could be kept at values close to normal by exerting rhythmic pressure over the sternum. However it cannot be concluded from this observation that the cardiac output is also close to normal. In fact, this is unlikely since the sternal compression also must raise pressure in the superior vena cava which in turn must disturb the venous return. A study of the possibility of maintaining circulation in the severely asphyxiated newborn by external cardiac massage therefore seemed essential. In view of the persistence of fetal shunts, assessment of cardiac output with indicator dilution techniques would have been difficult. Cinéangiography on the other hand, convincingly demonstrates how injected blood would be distributed through these shunts and how cardiac massage would influence this distribution.

Although the piglets we examined had no persisting heart activity our findings were very similar to those reported by other investigators studying lamb (1) or human fetuses (6) which were not breathing but had an intact circulation. Thus, the course of the contrast medium indicated that blood flowing into the right atrium through the superior vena cava was distributed to the right ventricle as well as to the left atrium. On the other hand, blood carried into the right atrium by the inferior vena cava passed mainly to the left atrium and left ventricle. Cardiac massage in all instances forced blood out into the arterial system.

Distribution of contrast medium in a heart at standstill might be influenced by a difference in specific gravity between blood and contrast medium. Therefore in the present study the piglets were randomly placed either on their left or their right side. There was no evidence that the distribution of contrast medium was affected by the position of the piglet.

In severe neonatal asphyxia infusion of oxygenated blood improves cardiac activity. This was found to be true when the blood was infused through the umbilical vein, and it was assumed that from this vessel the oxygenated blood would follow the fetal route, i.e. the short cut to the ascending aorta. The result of our angiographic study has proven this assumption to be true and it would seem to be more advantageous to inject the blood into the umbilical vein than into the jugular vein. By the latter route the blood would be distributed partly to the right ventricle and, through the ductus

arteriosus, it would reach the descending aorta and then be conducted away from the vessels of the target organs, the heart itself and the central nervous system.

The conspicuous and reproducible improvement of cardiac activity elicited by injection of oxygenated blood into the umbilical vein confirms our previous observations (3 4) When the infusion was made with contrast medium alone no heart activity was elicited but with cardiac massage it was possible to force blood from the heart into the arterial system. In case these observations are valid also in the human, it may be concluded that in severely asphyxiated human infants, infusion of oxygenated blood and external cardiac massage are methods worth trying.

ACKNOWLEDGMENT

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UTERO-VAGINAL DIFFERENTIAL PRESSURE AT REST AND DURING PHYSICAL EFFORTS IN EARLY PREGNANCY

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Abstract. Simultaneous recordings of the intra-uterine and intra-vaginal pressures were carried out in 24 women in early pregnancy. At rest the uterine pressure was slightly higher than the vaginal pressure. Body position and physical efforts resulting in increased intra-abdominal pressure had no essential effect on the utero-vaginal pressure difference. This would seem to be an important mechanism for protection of the ovum during pregnancy.

Schatz (2) was the first to measure the hydrostatic pressure in the uterus during delivery. He also noted (3) that the pelvic pressures are influenced by the intra-abdominal pressure. This does not yet appear to be generally accepted (1). A study of the utero-vaginal differential pressure in relation to uterine contraction during the first stage of labour was presented by Westin & Soderberg (6).

It would seem reasonable to assume that in early pregnancy the pressures on the two sides of the external cervical os are normally in equilibrium, regardless of changes in the intra-abdominal pressure produced by body position and physical activity. Otherwise there would be a risk of the ovum being expelled.

In order to test this hypothesis the following questions were posed.

1. Is there a utero-vaginal pressure difference at rest?
2. If so, is it related to body position?
3. Does an increase in intra-abdominal pressure during physical effort influence the utero-vaginal pressure difference?

MATERIAL AND METHODS

The investigation was carried out in consecutive series of 24 women in early pregnancy (11th–16th week of gestation), 11 of them were nulliparous and 13 parous. The women were patients at the Department of Obstetrics and Gynaecology, Sabbatsbergs Hospital, Stockholm, for intended therapeutic abortion. Pregnancy was normal. The membranes were intact and no drugs had been given. At rest the mean distance between the posterior commissure of the vulva and the cervix was, in both nulliparous and parous women, 6 cm (range: 5–8 and 4–12 cm, respectively).

The intra-uterine and intravaginal pressures were recorded simultaneously with two closed catheters and electromanometers. One catheter was placed with its tip between the uterine wall and the membranes and the other fixed with its tip in the vagina at the level of the external cervical os. Details of the method have been described elsewhere (4, 5). No contractions of the uterus or the vagina were observed during the recordings.

RESULTS

Pressures at rest

As shown in Table I, at rest the uterine pressures were slightly higher than the vaginal pressures both in the supine and the erect positions. However, the difference between the utero-vaginal pressure gradients in the supine position and those in the erect position was not significant either in nulliparous ($p > 0.05$) or in parous women ($0.05 > p > 0.01$). Neither was the difference between the utero-vaginal pressure gradients of nulliparas and those of paras significant either in the supine position ($p > 0.05$) or in the erect position ($0.05 > p > 0.01$).

Increases in uterine and vaginal pressures during coughing and straining

As shown in Table II, the pressure increases in the vagina and the uterus during coughing and straining were of the same magnitude both in

Table I *Pressures at rest (cm of water)*

Position	Parity	n	Uterus $\bar{X}$	Uterus 95% confidence interval of μ	Vagina $\bar{X}$	Vagina 95% confidence interval of μ	Significance of difference between pressures
Supine	Nulliparous	11	23	17–28	10	6–13	$p<0.001$
	Parous	13	24	14–34	10	8–13	$0.05>p>0.01$
Erect	Nulliparous	11	50	44–57	34	29–39	$p<0.001$
	Parous	13	55	46–64	33	28–38	$0.01>p>0.001$

the supine and the erect positions. In no instance was there a significant difference between the ratio of pressure increases and 100% either in nulliparous or in parous women.

DISCUSSION

In this study of early pregnancy the pressures in the uterus and the vagina showed a slight difference (about 15 cm of water) at rest in the supine position The pressure was higher in the uterus than in the vagina, which may be due to the muscle tonus of the uterus. In cases of cervical incompetence in otherwise normal pregnancies, the cervical dilatation could be attributable in part to such slight physiological difference in the utero-vaginal pressures.

In the erect position both the uterine and vaginal pressures were higher than in the supine position, as described already by Schatz (3) and shown recently (5) in full-term pregnancy However the utero-vaginal pressure gradient remained unchanged. This applied also during physical efforts resulting in increased intra-abdominal pressure.

The stable hydrostatic suspension seems to be an important protective mechanism for the ovum.

It deserves attention in the treatment of threatened abortion where a tolerant attitude toward mobilisation of the woman seems justified in cases where the cervix is well above an intact and well-functioning pelvic floor

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Table II. *Ratio in per cent between vaginal and uterine pressure increases during coughing and straining*

Activity	Position	Parity	n	$\bar{X}$	95 confidence interval of μ	Significance of difference from 100
Cough	Supine	Nulliparous	11	102	95–108	$p>0.05$
		Parous	13	104	9?–116	$p>0.05$
	Erect	Nulliparous	11	103	95–111	$p>0.05$
		Parous	13	101	95–106	$p>0.05$
Strain	Supine	Nulliparous	11	104	96–111	$p>0.05$
		Parous	13	95	86–105	$p>0.05$
	Erect	Nulliparous	11	105	97 114	$p>0.05$
		Parous	13	92	85–98	$0.05>p>0.01$

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MODIFIED UTERINE PHLEBOGRAPHY AND HYSTEROGRAPHY IN THE DIAGNOSIS OF UTERINE MYOMAS

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Abstract. The value of modified uterine phlebography (simultaneous phlebography and hysterography) was studied in 34 patients with clinical diagnosis of uterine myoma. All the findings were verified at operation, and 31 of the patients were found to have myomas. The group best suited for comparative purposes comprised 25 patients with intramural or submucous myomas. Uterine phlebography gave a quantitative diagnosis in 22/25 of these patients and hysterography in 10/25. Uterine phlebography failed to reveal the diagnosis in one case of cervical myoma. The diagnosis was qualitative in both examinations in the other cases. Hysterography was better for detecting submucous myomas, and uterine phlebography for intramural myomas. The results show that the information given by modified uterine phlebography and hysterography is complementary. The uterine phlebography instrument employed in the investigation requires further development.

An accurate preoperative diagnosis of the nature and extent of the disease is desired when planning surgical therapy of gynecologic diseases. Hysterosalpingography is important in the diagnosis of uterine myomas (4) and it is the most important diagnostic method for submucous myoma. An accuracy of almost 80% is achieved for intramural myomas when the diagnosis is based on deformity of the uterine cavity shown by hysterography. Studies of uterine phlebography have shown that this method is useful in diagnosing uterine myomas (1, 2, 5, 6). We therefore tried to diagnose uterine myomas using modified uterine phlebography (uterine phlebography combined with simultaneous contrast filling of the uterine cavity). The preliminary results were promising (3). The purpose of this study was to compare modified uterine phlebography and hysterography in the diagnosis of uterine myomas.

MATERIAL

The material consisted of 34 patients (range of age 30–53 years) who were subjected to laparotomy following the clinical diagnosis of uterine myomas. Myomas were found at operation in 31. Twenty-five patients had an intramural myoma or a multinodular myomatous uterus, three patients had solely submucous myomas and three patients subserous myomas only. No myomas were encountered at operation in three cases; two of these patients had a large ovarian endometrioma and one pelvic varicosities.

METHOD

Routine hysterography using Perjodal HD was performed first and then the modified uterine phlebography with a single-needle instrument. The mode of use of the uterine phlebography instrument has been presented earlier (2). The instrument used in this study had a second channel for filling the uterine cavity with contrast medium (Fig. 1). The centre of the uterine fundus was punctured by the needle under fluoroscopic control. During each exposure 20 ml of Angiografin® was injected through the needle and the uterine cavity was filled simultaneously with Perjodal HD from another syringe. The roentgenograms were made in the posteroanterior and in both oblique directions with an ordinary fluoro-

Table I. *The comparison between the hysterography and modified uterine phlebography in 25 operatively verified patients*

	Quantitative diagnosis	Qualitative diagnosis	Non-diagnostic	Total
Hysterography	10	15	—	25
Uterine phlebography	22	2	1	25



Fig 1 The instrument used for modified uterine phlebography The short arrows point to the second channel used to fill the uterine cavity The long arrows point to the needle used for phlebography

scopy unit. The examinations were usually done the day before the operation.

The roentgenograms were examined before the operation findings were known. The diagnostic significance of the roentgenologic changes was determined separately from the hysterograms and the roentgenograms obtained at modified phlebography The information was classified in the following way-

1) Quantitative diagnosis. Changes typical of uterine myomas which also indicate quite well the number size and location of the myomas.

2) Qualitative diagnosis. The finding is characteristic of uterine myomas but the size, number or localisation of the myomas cannot be established.

3) Non-diagnostic: The finding is either normal or there are changes which are not diagnostic for myomas.

RESULTS

Uterine phlebography failed in one case This was a patient with subserous myomas whose arterial network of the uterine muscle could not be found despite several punctures. Hysterography was not done in 4 cases.

Comparable results were available for 25 patients in which myomas were found at operation (Table I) They were intramural in 22 cases (Figs. 2–3) and submucous in three (Fig. 4). Comparison with the 3 patients with subserous and 3 with intramural myomas was inadequate because of incomplete facts.

The most important group comprised the 25 patients with intramural myomas or myomatous uterus. Three patients had no hysterography Both investigations gave a quantitative diagnosis in 7 cases. Hysterography gave only a qualitative but uterine phlebography a quantitative diagnosis for 13 patients. Of the rest, the uterine phlebography finding was quantitative in one case and qualitative in 4 cases, in 3 of which the myoma was cervical. In all uterine phlebography gave a quantitative diagnosis in 21/25 patients and a qualitative diagnosis in 4/25 patients of this group. The corresponding figures for hysterography were 7/22 and 15/22. In the three cases of cervical intramural myomas hysterography was diagnostic in all cases and uterine phlebography in two (Fig. 5) In the non-diagnostic case the veins in the uterine cervix filled inadequately



Fig A patient with an intramural fundal myoma. ( ) Hysterogram, in which the right cornu is deformed. (*b*) Modified uterine phlebogram shows the deformation of the uterine cavity and the outer surface and the size of the myoma (*arrow*).





Fig. 3. A patient with large degenerative intramural myoma in the dorsal part of the uterus. (*a*) Hysterogram with typical deformation and dislocation of the uterine cavity. (*b*) Uterine phlebogram shows also the size of the myoma and the dilated uterine veins.

The hysterographic diagnosis in all 3 patients with submucous myomas was quantitative. The modified uterine phlebography finding was quantitative for two of them and qualitative for one.

Contrast medium passed below the "myoma capsule" in six cases in uterine phlebography resulting in clear visualization of the myoma (Fig. 6). It appeared that for this the tip of





Fig. A patient with submucous myoma. (*a*) Hysterogram reveals the myoma in the left uterine cornua. (*b*) The myoma can also be seen in the modified uterine phlebogram. There is defect in the filling of uterine cavity. The uterine veins are not displaced.

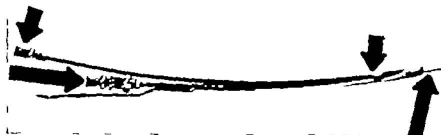


Fig. 1. The instrument used for modified uterine phlebography. The short arrows point to the second channel used to fill the uterine cavity. The long arrows point to the needle used for phlebography.

scopy unit. The examinations were usually done the day before the operation.

The roentgenograms were examined before the operative findings were known. The diagnostic significance of the roentgenologic changes was determined separately from the hysterograms and the roentgenograms obtained at modified phlebography. The information was classified in the following way:

1) Quantitative diagnosis. Changes typical of uterine myomas which also indicate quite well the number, size and location of the myomas.

2) Qualitative diagnosis: The finding is characteristic of uterine myomas but the size, number or localisation of the myomas cannot be established.

3) Non-diagnostic: The finding is either normal or there are changes which are not diagnostic for myomas.

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Fig. 2. A patient with an intramural fundal myoma. (*a*) Hysterogram, in which the right cornu is deformed. (*b*) Modified uterine phlebogram shows the deformation of the uterine cavity and the outer surface and the size of the myoma (*arrow*).



Fig 7 A patient with an ovarian endometrioma on the left side (*a*) Hysterogram shows dislocation of the uterus to the right; there is no deformation which could indicate myomatous changes. (*b*) Uterine phlebogram shows that the process in the left side is outside the uterine wall and the size of the uterus is normal

also a hysterographic qualitative diagnosis. The majority of our patients had large myomatous uteruses and therefore the changes were quite evident in both investigations.

The study shows that modified uterine phlebography is a better diagnostic aid than hysterography for intramural myomas. Hysterography again, is a better method of detecting submucous myomas. Conventional uterine phlebography cannot reveal submucous myomas. On the other hand, with modified uterine phlebography this is possible with the aid of the simultaneous filling of the uterine cavity. It appeared, that uterine phlebography cannot always reveal cervical myomas, because the intramural veins in the cervical area sometimes fill inadequately.

The results show that the information provided by hysterography and uterine phlebography is complementary. Further development of the instrument employed in modified uterine phlebography is therefore required. Incorporation of a mechanism that closes the cervical canal will improve the diagnostic possibilities.

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Fig. 5. A patient with cervical intramural myoma. (*a*) In the hysterogram lengthening of the cavity and cervical canal can be seen. (*b*) In the uterine phlebogram the veins around the myoma in the cervical area are filled.

the needle had to be firmly inserted inside the myoma itself and hence only myomas in the fundus were visualised. In these cases the myomas were also detectable by phlebography or hysterography. The diameter of these myomas varied from 1 to 5 cm (Fig. 6).

Contrary to the clinical diagnosis, no myomas were encountered at operation in 3 cases. Two of these patients had ovarian endometriotic cysts; both hysterography and uterine phlebography gave a correct negative result regarding myomas and were indicative of an ovarian tumour (Fig. 7). On the other hand, both hysterography and uterine phlebography were considered to give a false positive diagnosis of intramural myoma in the third case. This patient was found to have a pelvic varicocele.

In the total series, a correct negative result was established twice, a false negative result for uterine phlebography once in a case with submucous myoma, a false positive diagnosis was made in one case with both examinations.

The instrument used in the modified uterine phlebography has no part that closes the cervix. In consequence the filling of the uterine cavity was good or satisfactory in half of the cases but poor in the other half as the uterine cavity emptied during injection of contrast medium.



Fig. 6. A patient with fundal intramural myoma. There is contrast medium inside the layers of the myoma (*arrow*).

DISCUSSION

A preliminary study suggested the possibility that modified uterine phlebography might be capable of disclosing small intramural myomas which escape detection by hysterography. The hypothesis was based primarily on the fact that the uterine venous network visualised in phlebography reveals not only the direct changes caused by myomas but also the thickness of the uterine walls and the outer surface of the uterus. Simultaneous filling of the uterine cavity with contrast medium would then disclose the local changes in the thickness of the uterine wall which are suggestive of myoma. It was not possible from this series to conform this hypothesis definitely. It seems likely in many individual cases, but they in ol ed enough deformity of the uterine cavity to permit

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CASE REPORTS

SPONTANEOUS PERFORATION OF THE CAECUM FOLLOWING CAESAREAN SECTION

Report of a Case and Review of the Literature

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Abstract. A case of spontaneous perforation of the caecum following Caesarean section in a 21-year-old primigravida is presented. The literature is reviewed and contributory factors are discussed. A caecal diameter exceeding 9 cm is stated to be an important sign of imminent perforation.

Perforation of the caecum associated with local disorders (infections, cancer, foreign bodies) and with overdistension caused by obstruction of the distal colon has been described several times in the literature (Saeltzer & Rhodes, 1935, Rack, 1952). Spontaneous perforation of the caecum in connection with paralytic ileus is extremely rare, and only about 10 cases could be traced in the literature (Eckmann et al., 1958, Hirsch, 1961, Millar & Øvlsen, 1966, Yeo, 1967, Dumont & Dovy, 1967). Four of these cases developed after Caesarean section (Robertson et al., 1958, Millar & Øvlsen, Dumont & Dovy). The mechanism underlying this rare type of perforation, which occurs most frequently on the anterior surface of the caecum and 5 cm distal to the ileo-caecal junction, has been described by several authors (Saeltzer & Rhodes; Van Beuren, 1926, Wangensteen, 1955, Lowman & Davis, 1956). The following factors are said to be decisive for this site of predilection. An increasing pressure in the caecum impairs the blood flow and will give rise to the development of necrosis in the areas most poorly vascularized. Experiments on animals have shown that if pressures of 20 ml of water are maintained for more than 24 hours, necrosis can develop. In the human, pressures of 12 to 52 cm of water have been measured in connection with colonic obstruction. In two cases where perforation occurred, a pressure of 20 cm of water was measured. Presumably the existence of a competent ileo-caecal valve found in about 10% of cases, might contribute to the predominant rise in pressure in the caecum (Fleischner & Bernstein, 1950); the appearance of a cecocolic sphincteric tract may also be of importance. Furthermore, the thin walls and large diameter of the caecum are factors which may exert an influence.

As is the case in all other acute abdominal disorders arising during the puerperium, the symptomatology is fairly limited. A review of the four cases described reveals rise in temperature, increased pulse rate, increased abdominal distension and severe either constant or intermittent, pain in the right iliac fossa in all the cases, in one case there were signs of peritoneal reaction. In all the patients, flatus was passed on the day of perforation. None of them vomited. Two of the patients stated that their condition suddenly deteriorated. Survey radiographs of the abdomen revealed varying amounts of free gas and severe distension of the caecum. In a comprehensive study Lowman & Davis (1956) examined the diameter of the caecum in connection with obstruction. In 19 patients with obstruction (7 with perforation of the caecum and 12 with imminent perforation) they found caecal diameters varying from 9.0 to 16.5 cm. In a series comprising 100 normal subjects, the caecal diameter was found to vary in a bell shape about 6.5 cm after barium and air insufflation. In 97 cases the diameter was less than 9.0 cm. Hence, Lowman & Davis concluded that a caecal diameter of 9 cm would be an indication

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for prophylactic caecostomy In the cases de scribed in the literature, the treatment was palliative caecostomy and antibiotics. Three patients responded to this treatment, one died in septic shock. The mortality in connection with perforation of the caecum is stated to vary from 35% to 72% (Albers et al. 1956 Wangensteen Lowman & Davis) However Lowman & Davis found a mortality of 8% in the 12 cases where caecostomy was carried out because of suspected perforation Because of the rarity of this complication and the difficulties encountered in establishing the diagnosis, the following case history was considered to be of interest.

CASE REPORT

A 28-year-old primigravida, who had been well previously apart from appendectomy at the age of 9 years, was admitted at term, after an uncomplicated pregnancy with a breech presentation. Infusion 1 glucose 5% with 5 I.U. of synthetic oxytocin (Syntocinon) was given over a period of 48 hours in an attempt to induce labour without effect, and consequently it was decided to carry out a Caesarean section. A healthy female infant was delivered by Caesarean section with a transverse incision. Weight 3 400 grammes, length 52 cm. Apgar 10 was obtained after 1 min. The operation was uncomplicated. In the postoperative period, there was slight paralytic ileus for the first 72 hours, but flatus was passed. During the fourth and fifth days slight diffuse abdominal pain and increasing meteorism developed. Survey radiography of the abdomen on the fifth day revealed signs of paralytic ileus, a large amount of free gas under the diaphragm and, on repeated examination of the radiograph, a caecal diameter of 10 cm was seen. The temperature was 36.8–37.2°C, the pulse rate slightly raised, 92–120. Serum electrolytes normal. The abdominal distension increased alarmingly and her general condition deteriorated. Repeated survey radiography of the abdomen revealed unchanged paralytic ileus, but the volume of subdiaphragmatic gas had increased considerably Because of suspected bowel perf ration, explorative laparotomy was carried out. This revealed a severe diffuse peritonitis with numerous small abscesses in between the bowel and, on the anterior wall of the caecum, about 4–5 cm distal to the ileo-caecal valve, a 2 cm large perforation. An area of about 1.5–1 cm around the perforation was necrotic. After resection of necrotic tissue, the perforation was closed in two layers with atraumatic catgut and silk sutures. Treatment with antibiotics was instituted, and later adjusted according to the sensitivity tests. Subsequent complications included two subphrenic abscesses, several intestinal abscesses and several fistulas of the small intestine. After drainage, antibiotics and two additional laparotomies with closure of fistulas, the patient was discharged about 2 months after the Caesarean section. At this time there was still fistula of the small intestine with some discharge.

DISCUSSION

We cannot offer any explanation of why the patient, described in the present paper suddenly developed an aggravation of the paralytic ileus 4 to 6 days after operation. No signs of organic colonic disease were seen at operation, and there was no evidence that the uterus was obstructing the sigmoid colon (Cannell & Tovee, 1957 Antony & Wallace 1962)

The enema given on the fifth day for therapeutic purposes might have affected the caecal pressure and, thereby the course of disease, because a pressure of about 100 cm of water is commonly used to introduce an enema, and this pressure might be transmitted partly to the caecum.

CONCLUSION

In cases of paralytic ileus associated with dilation of the caecum, perforation should be suspected if the caecal diameter exceeds 9 cm. Enemas as a therapeutic measure are contra-indicated under such circumstances.

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ULTRASTRUCTURE OF A HUMAN IMPLANTATION SITE

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Abstract. The fine structure of the trophoblast from previllous human ovum is described. The extra-embryonal mesoderm was lined by cytotrophoblasts, which were beginning to form the primary villi The ultrastructure of these cells is similar to that of the Langhans' cells of the definitive villus The syncytiotrophoblast was located peripherally and consisted of mixture of giant cells and syncytial masses This cell type was well differentiated, with its cytoplasm occupied by large amounts of granular endoplasmic reticulum, Golgi elements, and vesicles The syncytiotrophoblast had many microvilli and pinocytotic vesicles at the surface, lining the lacunae which contained maternal blood cells Close contact was observed between the trophoblast and the cells of the maternal glands The trophoblastic invasion of maternal vessel described, and the trophoblastic invasion is discussed.

Many cases of infertility and early abortion may result from defects in the implantation of the blastocyst, and this process therefore deserves the interest of the clinician The early stages of implantation take place during the second week after ovulation Therefore defective implantation may not be diagnosed if the bleeding following the destruction of the conceptus occurs at the time of the expected menstruation

Moreover additional information about the implantation process may result in development of contraceptives acting at this stage of reproduction

Light microscopy of the human implantation site was studied in the series of conceptuses described by Hertig, Rock & Adams (6) and Hamilton & Boyd (3) Although the ultrastructure of the implantation site has been described in a number of species, the human implantation process has not been studied previously with the electron microscope. The earliest human material studied ultrastructurally is from the 22nd day of gestation (9)

MATERIAL AND MEHODS

Case history

The patient was 24-year-old II para IV gravidae. Hysterectomy was planned because of "pelvic congestion syndrome" and constant fear of pregnancy She suffered from chronic pelvic pains and dyspareunia and had contraceptive problems (recurrence of endometritis after insertion of an intrauterine device and tendency to depressions on oral contraception)

During control cycle the patient measured the basal body temperature Assays of plasma progesterone and LH in the urine wer also carried out (Table I). It was estimated that the ovulation took place between the 15th and the 17th day of the cycle. Therefore, it was decided to perform the operation on the 25th day of the next menstrual cycle Fig 1 show the basal body temperature during the week before the operation This indicates that the ovulation took place on the 14th or 15th day of the cycle and the patient reported that she had intercourse on the 10th, 13th, 14th, and 18th day

A subtotal hysterectomy was performed The uterine arteries were clamped immediately before removal of the specimen to minimize ischaemia. The uterine cavity was opened along the paramertrial margins, and surface fixation with 4% glutaraldehyde as started within 3 sec of the interruption of the blood supply The endometrial surface as now searched under zoom-stereo microscope while the surface was moistened with fixative.

The implantation site was observed as small red ring on the posterior wall, 10 mm to the *right* of the median and equidistant between the fundus and the isthmus After initial identification, it was possible to see the ring with the naked eye, as its diameter was approximately 0.8 mm. At higher magnification (30), the red ring could be resolved into numerous small red dots.

After photography the implantation area was removed

ler of maternal erythrocytes. The embryo was bilaminar and located at the deepest portion of the blastocyst (Fig. 2)

The trophoblast consisted of cytotrophoblast covered by syncytiotrophoblast. The latter consisted of a mixture of larger syncytial masses and giant cells of syncytial type. No villous formation was observed.

The *cytotrophoblast* (Figs. 3 and 6) had one large regular nucleus with skein-like nucleoli. The rather undifferentiated cytoplasm contained few mitochondria, of which most were regular though irregular types were also present. Furthermore, the cytoplasm had a single Golgi complex and many free ribosomes, whereas elements of rough surfaced endoplasmic reticulum were rare. Some glycogen deposits were encountered. Numerous desmosomes with adjacent cytotrophoblasts and with the syncytiotrophoblast were found.

The *syncytiotrophoblast* (Figs. 4 and 5) had large, irregular nuclei with deep indentations. Often more than one nucleolus could be seen. Although many free ribosomes were observed, the cytoplasm was dominated by rough-surfaced endoplasmic reticulum which consisted of uniform vesicles containing homogenous material of low electron density. Numerous communications with the elements of the well developed Golgi complexes (Fig. 5) were observed. The mitochondria were uniformly shaped with well defined cristae. Many inclusions (Fig. 5) were observed in the cytoplasm. They varied in shape and electron density, some being myelin figures (Fig. 4), some having single membrane-bound granular content. Lysosomes were also seen (Fig. 5). A few phagocytosed erythrocytes could be found. Sometimes, bundles of microfibrils (Figs. 4 and 5) were seen in the cytoplasm in which, also, few isolated desmosomes appeared. The cell membrane, facing the intervillous space (Figs. 4 and 5) possessed many slender microvilli on its often irregular surface. Numerous micropinocytotic vesicles were present (Fig. 5)

The trophoblastic lining of the extra-embryonal mesoderm (Figs. 2 and 6) consisted of continuous layer of undifferentiated cytotrophoblasts with an inconspicuous basement membrane. No basal infolding, pinocytotic activity or dips between the cells from the covering syncytiotrophoblast were encountered.

The *border line* between the invading trophoblast and the endometrium was somewhat irregular often with blunt, irregular processes (Fig. 7). The cytoplasm showed abundant fibrillar material just below and parallel to the surface membrane. In many places, the connective tissue cells were seen in close contact with the trophoblast. However an intercellular space was always found between the two kinds of cells, and localized close contact was never observed. Accordingly surface membrane specializations (e.g. desmosomes) were never seen between trophoblast and stroma cells. By accident, trophoblastic cells isolated in the maternal stroma were observed. Some infiltration with small and medium-sized lymphocytes in the stroma was encountered (Fig. 2)



Fig. 3. Portion of cytotrophoblasts connected by desmosomes (d). Arrows point towards syncytium. Note the uniform cytoplasm with abundant free ribosomes. Mitochondria (m). Golgi area (g). × 13 300. Figs. 3, 4, 5, 6, 7, 8, 10, and 11 are electron micrographs.

A uterine gland was found partly surrounded by syncytial trophoblast (Fig. 8). The basement membrane of the glandular epithelium intervened between the trophoblast and the glandular cells in most places (Fig. 9). However, in a few areas, the basement membrane had disappeared, and

Table I *Hormone assays*

Day of menstrual cycle	22	23	24	25
LH imm. I U./24 hrs	500	4 500	500	—
Plasma progesterone μg/ml	—	12.8	—	2.0*

* After removal of the corpus luteum.

in one block, measuring 2 2 × 1.5 mm, and fixed in 4% glutaraldehyde in a collidine buffer for total period of 1 hour After washing in collidine buffer and postfixation for 1 hour in % osmium tetroxide, the tissue was dehydrated through ethyl alcohol and toluene passages of increasing concentration, and embedded in Dow Epon.

After embedding, an attempt was made to cut the material symmetrically into halves by a vertical section. However survey sections revealed that the ovum was not bisected exactly Therefore a survey illustration of the specimen had to be made as a montage (Fig 2) from several fragments f the specimen, some of which had to be reembedded.

Thin sections were cut on an LKB-ultramicrotome and stained with aqueous solutions of uranyl acetate and lead citrate or with hot uranyl acetate in a 95% alcoholic solution (10).

The tissue was studied in JEOL JEM 6 C and Siemens electron microscopes.

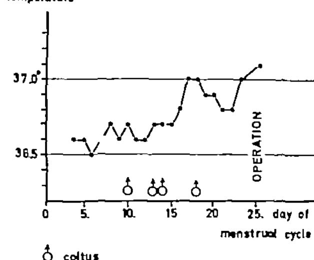


Fig 1

RESULTS

The ovum was found beneath the surface epithe lium which was in the process of closing the implantation gap The trophoblastic lacunae had formed and they contained a moderate num-

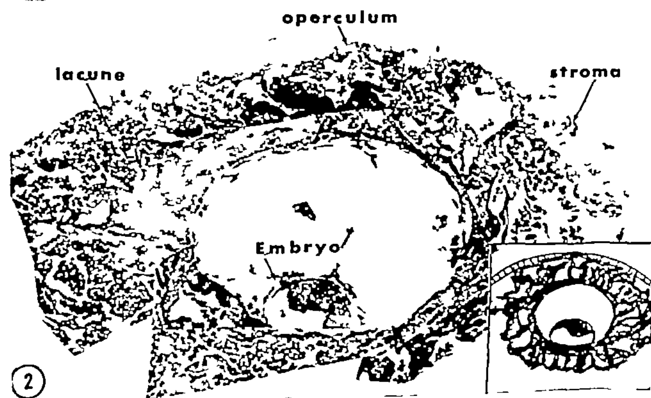


Fig 2 Light micrographic survey-montage of the implanted ovum. Approximately 75

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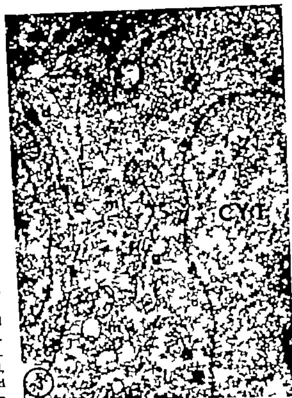


Fig 3 Portions of cytotrophoblasts connected by desmosomes (*d*) Arrow point towards syncytium Note the uniform cytoplasm with abundant free ribosomes. Mitochondria (*m*). Golgi area (*g*). 13 300 Figs. 3 4, 5 6, 7 9 10, and 11 are electron micrographs.

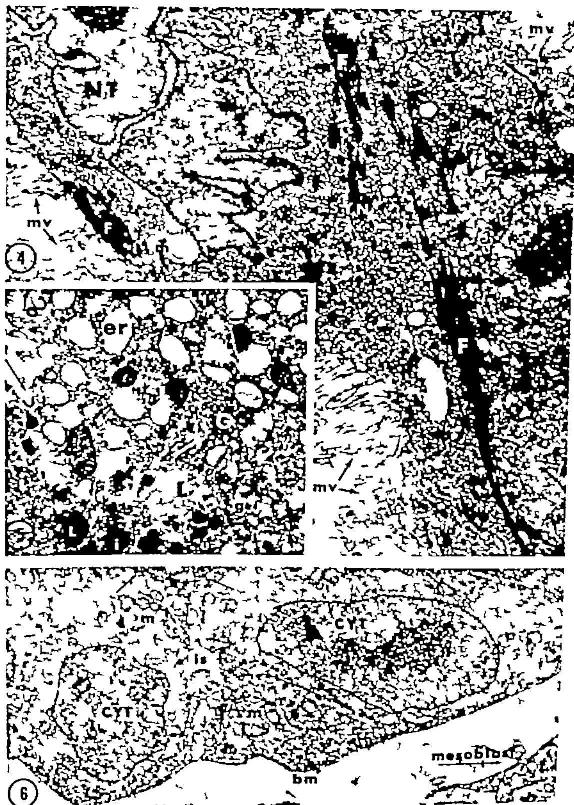


Fig 4 Portion of syncytial mass covering cytotrophoblast (*CYT*). Note the large, irregular syncytial nucleus (*NT*) and the cytoplasm dominated by vesicles. Long bundles of fibrillar material (*F*). The cell surface presents numerous microvilli (*mv*). Myelin figure (*m*) 6 000.

Fig 5 Portion of syncytial cytoplasm with microvillous cell surface at upper left edge. Pinocytosis at arrow. Granular endoplasmic reticulum (*er*) is seen as nuf vesicles. Golgi area (*G*). Mitochondrion (*m*). Lys (*L*). Inclusion (*i*). Fibrilles (*f*). Glycogen (*g*). 17 000

Fig 6 Cytotrophoblasts (*CYT*) lining the extraembryo mesoderm. Basement membrane (*bm*). Short arrow cat the covering syncytium. Intercellular space (*is*). Mit chondria (*m*). 6 700.









Fig. 7. Edge of advancing trophoblast (*SYN*). Note the relatively straight border with irregular processes. Maternal stroma (*St*) with portion of fibroblast (*FIBR*) showing short granular endoplasmic reticulum membranes. Degenerative area with myelin figure (*d*). × 10 500.

Fig. 8. Light microscopy showing uterine gland (*GL*) partly surrounded by syncytial trophoblast (*T*). Maternal stroma (*St*). Lacunae with erythrocytes (*LAC*). Framed area, see Fig. 9. × 450.

Fig. 9. Glandular epithelial cell (*EPIT*) with surface in upper right corner. Note the close apposition between epithelium and trophoblast (*SYN*), although the basement membrane (thin arrows) is intact. Collagen fibrilles (thick arrows). Tubular mitochondria (*m*), Golgi areas (*g*), degenerative areas (*d*). × 7 700.

Fig. 10. Survey electron micrograph showing syncytium pushing on maternal vessel. Close apposition between trophoblast and endothelium (between arrows). Note extravasated erythrocytes (*e*) in vicinity of vessel and interposed between part of trophoblast and vessel wall. Endothelial cell (*END*). Fibroblast (*fibr*) in maternal stroma. × 3 800.

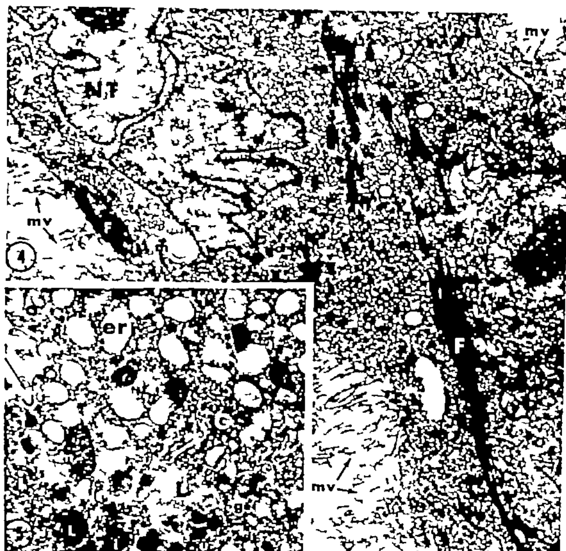


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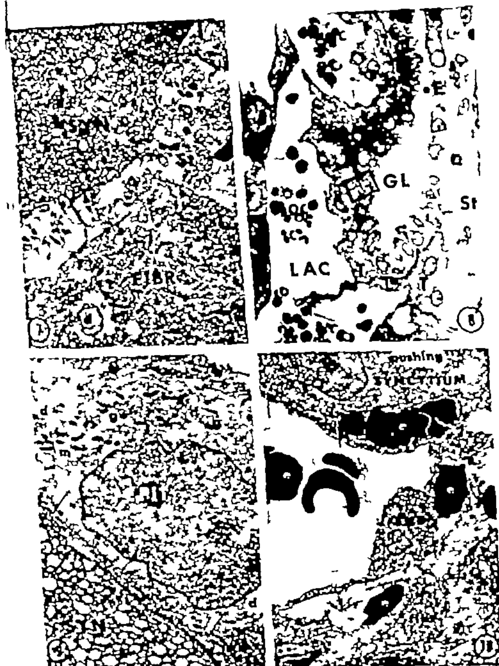


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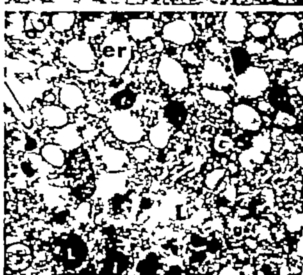






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Physiologic aspects

The present study confirms the rapidity of trophoblastic differentiation during the first few days. As the nidation process starts during day six after fertilization (4), the present ovum is in its fifth day of implantation. The differentiation of the syncytial trophoblast, evaluated by its fine structure, is already well defined as it is similar to that found in early chorionic villous syncytium (7) as well as that of the early trophoblastic giant cell of the syncytial type in the basal plate (9). The morphologic development of the organelles basically required for synthesis of glycoprotein, i.e. rough-surfaced endoplasmic reticulum and Golgi complexes, seems fulfilled for the increasing chorionic gonadotropin hormone production.

The pinocytotic activity on the microvillous surface of the syncytium lining the lacunae indicates the main pathway for nutritive uptake and excretory or secretory activity, whereas the surface towards the maternal endometrium appears much more passive in this respect. This seems to indicate that the nutritive function of material derived from endometrial debris is less important, at least at this stage. The digestive glycogen (4) could as well be of the ingestive type, i.e. glycogen from the deposits in the cytotrophoblasts that eventually fuse to form syncytial masses. Morphologically there is no difference between the two types of glycogen.

The fine structure of the cytotrophoblasts is similar to that of the Langhans cells of the definitive villus. Thus, these cells have a relatively undifferentiated appearance. This observation support the theories that the main function of the cytotrophoblast is to be progenitor of the syncytium (7, 9, 11).

The relation of trophoblast and maternal epithelium

Although the penetration through the surface epithelium by the trophoblast has been accomplished already, the finding of gland partly surrounded by trophoblast allowed observations on the trophoblast–epithelial junction zone.

As in the armadillo (1), the syncytium was separated from the gland cells by their basement membrane. However, occasional desmosomal contact between the two cell types was seen in areas where the basement membrane had disappeared. The epithelium shows a slight degree of degeneration in the human material, whereas in the guinea pig and the armadillo Enders & Schlaffke (1) saw viable looking epithelial cells next to the trophoblast.

The significance of the desmosome formation between trophoblast and the basis of the gland cells is uncertain. Trelstad et al. (12) in their study of cell contact during early morphogenesis in the chick embryo find it likely that focal tight junctions are points of transient cell adhesion which aid in cell migration. In their study of the penetration of trophoblast through the luminal epithelium, Enders & Schlaffke (1) suggest that attachments by surface specializations provide fixed points from which the trophoblast might flow thereby getting a specific direction. If the trophoblastic invasion of the glandular epithelium happens by a "flow" in between the epithelial cells, it seems likely that a point of fixation could be needed. However, evidence of invasion into the glands was not seen in the present study.

Morphologic evidence of mode of invation

Enders & Schlaffke (1) claimed that the trophoblast seems noncytolytic because adjacent epithelial cells look healthy even where glands are surrounded by trophoblast. On the other hand, they consider it possible that the trophoblast is histiolytic, producing breakdown of the intercellular components, and the eventual lysis of the epithelium in species which show extensive invasion such as the guinea pig and the armadillo, is an indirect action of the trophoblast by such alterations in the stromal matrix. This concept is valid in the present context, also. However, the trophoblastic surrounding of the glands per se when extensive must cause impairment of the blood supply of the epithelium. Since the epithelial origin of basement membranes is considered generally valid (2), the disappearance of the glandular basement membrane may be the expression of epithelial malfunction before signs of degeneration in the cells per se can be observed. If this is so, it explains the observation that trophoblast is lying adjacent to a row of gland cells for some time instead of a localized penetration. This disappearance of basement membrane was also described by Enders & Schlaffke (1) in the species mentioned.

A morphologic study cannot reveal release of possible enzymes involved in the histiolytic ac-

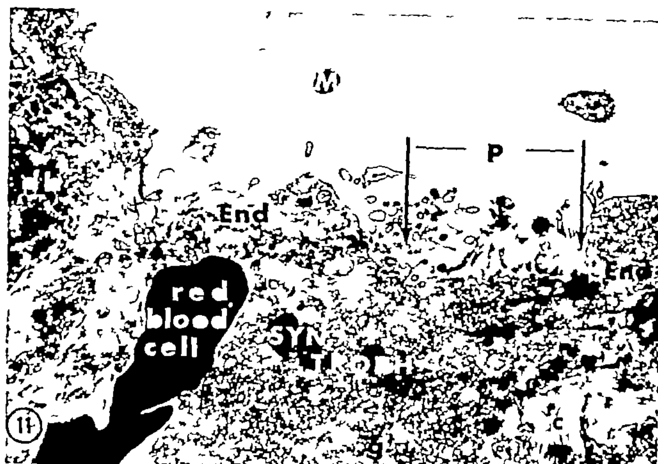


Fig 11 Portion of maternal vessel (*M*) with endothelium (*End*). Between arrows syncytial trophoblastic penetration (*P*) The surface of the trophoblast show microvillous border Collagen ( ). Golgi area (*g*) Debris (*db*). 6 900.

trophoblast was seen establishing desmosomal contact with the epithelium. The epithelium exhibited evidence of initial degeneration (Fig. 9) Fusion between trophoblastic cells and maternal cells was not observed.

In many places, maternal blood vessels were found closely related to the invading trophoblast, which was pushing on the endothelial wall (Fig. 10). In some cases, the endothelium was substituted partly by trophoblastic cell lining where the vessel lumina were in continuity with trophoblastic lacunae. Actual penetration by the trophoblast into a maternal vessel was only observed in one section (Fig 11). Extravasated blood cells were common findings in the vicinity of areas with close trophoblast-blood vessel relationship (Figs. 10 and 11)

DISCUSSION

Estimation of specimen age

The basal body temperature graph (Fig. 1) indicates that ovulation took place during the 14th or 15th day of the menstrual cycle. The patient had intercourse on the 14th day Therefore it seems likely that the fertilization took place during the 14th or 15th day As the operation was performed on the 25th day the ovum should have an ovulation age of 10–11 days.

Comparison with early implanted ova, the Carnegie embryos 7699 and 795 (6) and the Barnes embryo (3) indicates that the ovulation age is 11 days. This estimate is based on the following. The ovum was previllous, with intercommunicating lacunar spaces containing a moderate number of maternal erythrocytes. The cytotrophoblastic inner cell mass was beginning to proliferate (to form the primordia of chorionic villi) The amniotic cavity was well defined but the primary yolk sac was not so easily seen (Fig. 2)

In addition the red colour from small dots (the lacunae containing maternal blood) and the size (0.8 mm) correspond to the gross appearance and dimension of the implantation site of previllous ova (5).

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tivity of the trophoblast. The mode of invasion in relation to active breakdown of the intercellular matrix can only be guessed upon. However there seems to be no doubt that the increase in size of the implantation area at this early age is due to the expansive growth of the confluent trophoblastic mass (4) although on the other hand this expansion may be eased by the hormonal-induced edema of the stroma. This concept may be supported by the relatively even edge of the advancing trophoblastic bulk although this shows minor blunt protrusions. Thus, this observation is in agreement with the "pushing theory (9) and is best illustrated in the trophoblast–endothelial relationship in this work.

Origin of isolated trophoblastic giant cells

The syncytial trophoblast occupies the peripheral position of the advancing trophoblastic mass, whereas the cytotrophoblast is located centrally The formation of the trophoblastic shell during the following period is preceded by the development of the cytotrophoblastic villous stems, from the tips of which cytotrophoblastic cells migrate into the decidualizing stroma (3). This was shown by Larsen & Knoth (9) in their study of the fine structure of the anchoring villi from a four-somite embryo in which the proliferating cytotrophoblasts presented differentiation towards trophoblast of the syncytial type. The finding of isolated trophoblastic cells in this work is difficult to understand if they are not merely accidental break offs from the edge of the main mass of the advancing syncytiotrophoblast. Their fine structure is similar to that of the syncytial giant cells that are found in the syncytiotrophoblastic mixture Although Hertig (4) claims that during early basal plate formation he has seen the actual desquamation or casting off" of the remnants of syncytiotrophoblast basal plate formation has not started in this early ovum

The relation between trophoblast and maternal vessels

The fact that trophoblast was scaught in the act of penetrating a blood vessel in only one section in spite of examination of all the material available is somewhat surprising. This might indicate that this process in the human where the trophoblast is highly invasive is very rapid. In the rabbit, Enders & Schlaffke (1) saw primitive junctional complexes between the penetrating trophoblast and endothelium. No surface contacts to endothelium was observed in the penetration of trophoblast of a maternal blood vessel in the basal plate of a four-somite human embryo (Larsen & Knoth unpublished observation). On the contrary the trophoblastic cell presented a microvillous surface completely free of the adjacent endothelial cells.

In the present study the trophoblast is observed to push" on the vessel wall. No basement membrane seems to intervene. The penetration of the endothelium might be rather localized, in contrast to the breakthrough of the glands.

The blood sinuses, in this work, were found in several places having part of their endothelial lining substituted by trophoblast. A similar situation was described by Larsen (8) in the rabbit. Apparently the endothelium subsequently disappears as these blood spaces are incorporated in the advancing trophoblast.

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Announcements

During 1973 two courses on clinical cytology will be arranged at the Department of Obstetrics and Gynaecology University of Liège Belgium, under the patronship of the European Federation of Cytology Societies.

The first course taking place on 14–16 March 1973 is dedicated to the cytology of epidermoid epithelioma the second one on 12–16 November 1973 deals with vaginal hormonal cytology

For details, please write Docteur Andrée Pelzer Laboratoire de Cytologie Clinique Gynécologique et Obstetricale de l'Université de Liège 81 Boulevard de la Constitution B-400 Liège Belgium.

The Second International Symposium on Cancer Detection and Prevention will be held in Bologna on April 9–12, 1973

The Symposium is planned to discuss and evaluate the latest results in the field of cancer detection and prevention and held und[er the] auspices of the International Union [against] Cancer (UICC) and the International Agen[cy for] Research on Cancer (IARC) of the World H[ealth] Organisation.

Secretariat Istituto di Oncologia "F A[ddarii"] Viale Ercolani 4/2, I-40138 Bologna, Ital[y]

IX Acta Endocrinologica Congress will b[e held] in Oslo June 17th–21st 1973

President Dr Jørgen H. Vogt, Medical [Depart]ment B Aker Hospital, Oslo 5

Secretary General Dr Asbjørn Aakvaag [Hor]mone & Isotope Laboratory Aker H[ospital] Oslo 5

Enquiries should be addressed to the Se[cretary] General.

Books to Be Recommended

Post-Partum Intra-Uterine Conception in Singapore edited by D Wolfers. Excerpta Medica, Amsterdam 1970. 193 pages.

An important contribution concerning the use of IUD Of special interest are the good results obtained with an early insertion immediately after pregnancy Careful follow-up.

Barnpatologi, by Björn Ivemark. Almqvist & Wiksell, Stockholm 1971 332 pages.

Swedish text. An excellent textbook for undergraduates, which, however also can be used in postgraduate teaching.

Hermaphroditism, Genital Anomalies and Related [Endo]*crine Disorders*, by Howard W Jones Jr and Wallace Scott. The Williams & Wilkins Company [Balti]more 1971 564 pages.

A modern, excellently illustrated textbook co[vering] embryology diagnosis and treatment of genital [malforma]tions and related endocrinal disturbances.